

The Control of Teliospore and Urediniospore Formation by Experimental Methods

CHARLES W. WATERS

A Dissertation

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy
in the University of Michigan,
June, 1927.

[Reprinted from PHYTOPATHOLOGY, February, 1928, Vol. XVIII, No. 2]

Ann Arbor



THE CONTROL OF TELIOSPORE AND UREDINIOSPORE FORMATION BY EXPERIMENTAL METHODS¹

CHARLES W. WATERS

INTRODUCTION

The significance to be attached to the formation of teliospores² following production of urediniospores in the rust-fungi has been problematical ever since this very important group of parasitic fungi has been investigated by botanists. The assumption that it is an innate characteristic which must find its expression during the course which the fungus takes in its development in the host has long been shown to be insecure, not only by common observation but by studies on the subject such as those attempted by Iwanoff (18), Morgenthaller (28), Gassner (14), Smith (40), and others.

These investigators have attempted to show by experimentation and observation that the telial generation of the rusts stands as an expression of the ability of the fungus-parasite to react to certain environmental conditions unfavorable for its continued vegetative development and to produce a fruiting structure which will tide the fungus over such adverse conditions.

Among the various causes that have been suggested as stimulating the production of such a resistant stage are direct climatic influence, temperature, moisture, resistance of the host, inner organization of the fungus, indirect climatic influences acting through the host, and so forth. However, in practically all of the experiments and observations that have been made in the past, there has been no serious effort to get at the bottom of the problem and attempt, by correlation of the results which the various factors produce, to deduce some fundamental principle which might underlie and govern the appearance or non-appearance of the teliospore generation in the rusts.

It has been the aim of this work to determine such an underlying principle and, in this paper, to analyze the results obtained and their relation to such a principle.

¹ Papers from the Department of Botany of the University of Michigan, No. 271.

² Since the terminology of the spore forms as suggested by Arthur (4) is gradually replacing the old, the former will be used throughout the paper.

The work was done in the Cryptogamic Laboratory of the University of Michigan under the direction of Professor C. H. Kauffman, to whom the writer is greatly indebted for generous advice and helpful criticism.

EXPERIMENTAL RESULTS

In the following work ten species of rusts were used: *Uromyces appendiculatus* Fr., on *Phaseolus vulgaris* L.; *Puccinia taraxaci* Plowr., on *Taraxacum officinale* Weber.; *Puccinia suaveolens* Rostr., on *Cirsium arvense* (L.) Scop.; *Puccinia sorghi* Schw., on *Zea mays* L.; *Puccinia asparagi* DC., on *Asparagus officinalis* L.; *Puccinia tritici* Eriksson, on *Triticum vulgare* Vill.; *Uromyces trifolii* Lev., on *Trifolium hybridum* L.; *Puccinia antirrhini* Diet. and Holw., on *Antirrhinum majus* L.; *Uromyces polygoni* Fuckel, on *Polygonum aviculare* L.; and *Puccinia orbicula* Pk. and Clint., on *Prenanthes alba* L.

Experiments were conducted with these rusts both on the hosts grown in pots in a greenhouse and on leaves or portions of leaves of the hosts grown on solutions in petri dishes in the laboratory.

Technique

In the Greenhouse

In all cases when the healthy host plants were brought in from the field and infection was obtained, checks were maintained and remained healthy. The various hosts were cultivated in pots in the greenhouse and infection obtained in a manner similar to that suggested by Melhus (26) and Fromme (12), that is, spore suspensions were made in distilled water and sprayed on by means of a No. 16 De Vilbiss atomizer, after which the plants were placed in a moist chamber for 48 hours at a temperature varying from 17° to 22° C., depending on the rust. Variations of this method were used at times, such as first spraying the plants with distilled water and then applying the inoculum with a scalpel or camel's-hair brush as advocated by Carleton (7). The latter method insures a heavier infection, but if one desires a uniform infection for comparative studies the former method is the more satisfactory.

In working with plants such as alsike, wheat, or corn, the waxy coating of the leaves prevents the spore suspension from adhering. In such cases it is necessary to draw the leaf carefully between the moistened thumb and fore-finger before applying the suspension (Melchers, 25).

In the greenhouse, various incubation chambers have been employed. Under the ordinary conditions met with during the greater part of the year, a Wardian case and bell jars have been found to be the most satisfactory. In the summer, however, it was found to be almost impossible to infect the

plants successfully by these means alone, due to the prevailing high temperatures. The "iceless refrigerator," as suggested by Hunt (17), solved the difficulty. In the refrigerator, with modifications, infection was obtained with as much ease in the hottest part of the summer as at any other time of the year. It has been found by the writer that, on a day when the temperature registered 35° C. in the greenhouse, by means of the "iceless refrigerator," it was possible to keep the plants at a temperature not exceeding 26° C. This was accomplished by opening the ventilators of the greenhouse in such a way that a breeze was constantly blowing over the surface of the refrigerator, thus increasing the evaporation and lowering the temperature. It was found, by constructing the refrigerator of sufficient size to enclose the Wardian case, that the humidity was kept higher and the plants became more heavily infected than when they were left uncovered in the refrigerator. During the spring and fall of the year when the temperature was not sufficiently high to warrant the use of the refrigerator, a metal pan was used as a cover for the Wardian case. This was kept filled with water and lowered the temperature of the case sufficiently for infection.

In applying the inoculum to the leaves of the various hosts, a difference was found in the amount of infection produced, depending upon which surface received the spores. As the stomata are, so far as we know (Ward, 46) (Evans, 11), the only means of entrance for the germ tubes of the urediniospores, the amount of infection resulting from the inoculation will vary directly with the number of stomata.

In working with infection on hosts such as corn and wheat, in which the leaves assume a more or less vertical position, the writer has been unable to find a correlation between the amount of infection and the surface receiving the inoculum, although Melchers (25) reports one-half as many stomata on the lower surface of wheat leaves as upon the upper. However, on hosts such as bean, alsike, snapdragon, etc., it was found that more severe infection was produced by inoculation on the lower surface. The snapdragon shows the greatest degree of variation, especially when leaves were inoculated in petri dishes. In the first part of the work the snapdragon leaves were always inoculated with the upper surface uppermost. The result was a very slight infection. Finally, leaves were placed in the dishes in a reversed position and the spores applied to the lower surface. Two days later the position of the leaves was changed, so as to increase photosynthesis, and just before the time for the uredinia to break through the epidermis they were again placed in the position which they occupied at the time of inoculation. Heavy infection resulted from such procedure. In all subsequent inoculations of snapdragon leaves in petri dishes, therefore, the spores were applied to the lower surface. The ideal arrangement for producing infection in petri dishes would be to place the leaves with their lower surfaces

up and have the light enter from below. This was tried by means of electric lights with noticeably increased infection. But, owing to the difficulties attending such an arrangement, it was not employed in any of the recorded experiments.

In working with plants in the greenhouse during the period of short days, the light factor became increasingly more difficult to control. With plants such as corn, beans, snapdragon, and wheat, no special difficulty was encountered. With the other hosts, however, such as alsike, Canada thistle, *Taraxacum*, and *Polygonum*, during the short days of December and January, it was almost impossible to maintain the rusts. It was only through the added influence of electric lamps, suspended a few feet above the pots, that the writer was able to keep the last-named rusts in a condition suitable for experimentation.

In addition to the difficulties that were encountered with respect to light, there arose other factors that were detrimental to the best development of the rusts. Powdery mildew made its appearance from time to time on the dandelion, alsike, wheat, bean, and *Polygonum*, and was only with difficulty controlled. Once a plant became infected with rust it was almost impossible to eliminate mildew without also killing the rust. However, by inoculating the plants with the rust and then allowing several days to elapse to insure entrance of the germ tubes, the leaves could be safely sprayed with a 1-1000 aqueous solution of sulfuric acid. The plants were later rinsed with water, and by the time the uredinia broke through the epidermis the leaf was practically free from mildew. Fromme (12) reports using this method with sulfur dust 24 days after inoculation.

In addition, insect pests added other difficulties. With the advent of the various leaf hoppers, white flies, thrips, and red spiders, the rusts at times encountered almost overwhelming competition. As it is an established fact that a vigorous host is necessary for a successful rust infection, it is essential in working with the rusts to have a good supply of healthy vigorous host plants. To obtain them, however, is not an easy matter in the average greenhouse.

In Petri Dishes

In inoculating leaves in petri dishes, the same methods were used as in the pot inoculations. In preparing leaves for inoculation, care was taken to reduce to a minimum contamination from extraneous fungi.

The leaves were first removed from the host plant and immediately placed in a dish of water. They were then washed for several minutes in running water, rinsed in several changes of distilled water, and finally placed in a dish of sterile distilled water until ready for use.

The petri dishes were sterilized in the hot-air oven in the usual manner and the leaves placed therein. In the early experiments the solutions were

always placed in the dishes at the same time as the leaves. Later experiments proved, however, that better infection resulted if the spores were sprayed on the leaves and just enough water added to the dishes to keep the air saturated for 48 hours; at the end of this time the solutions were added and the petioles of the leaves cut back in order to facilitate intake of the nutrient.

Various methods of placing the leaves in the dishes were tried out. The first method used was to place glass rods in the bottom of the dishes and allow the leaves to rest upon them. The solution was then added in such amounts that the leaves did not come in contact with the solution except for the immersion of the petiole. Another method tried was to cut circular discs of screen wire with the edges bent down in order to raise them to the proper height for the dishes and then dip them in melted paraffine to prevent rusting. The leaves were laid upon these wire discs with the petioles extending down through one of the openings into the solution. With these methods, leathery leaves became infected, but tender leaves usually died before infection took place.

Clinton and McCormick (8) used a method somewhat similar to this: rubber bands were stretched across the dish and the leaves placed upon them. These investigators encountered the same difficulties as the writer in attempting to infect delicate leaves.

The method finally used was to float the leaves freely upon the solution, either sugar or distilled water. No leaf has yet been tried which could not be kept alive and in good condition for five or six weeks with this method. When floating leaves on sugar solutions, from which the leaf is to absorb nourishment, the more cut surfaces exposed to the solution the greater the amount and the more rapid will be the absorption. This can be easily demonstrated by clearing the leaves and applying the iodine test for starch, for it is this form of carbohydrate into which the sugar is transformed for storage in most leaves. This has been shown very beautifully by Saposhnikoff (35), Acton (1), Tollenaar (44), and others. It appears that the starch is deposited centrifugally from the vascular bundles outward, the more distant cells showing the starch last. When the cut surfaces are exposed the sugar apparently travels from cell to cell by diffusion but more slowly than through the bundles. Various nutrient solutions were used upon which to float the leaves, but it was found that a five to seven per cent sucrose solution gave the best results. Ordinary commercial cane sugar was used in all the experiments, and no attempt was made to secure a chemically pure product. It was found in the course of the work that not only did the sucrose solution prove to be the form of nutrient that was best utilized by the various leaves, but it remained uncontaminated for a much longer period of time. Especially was this true of the unsterilized solution.

At first, care was taken to sterilize the solution of sucrose before it was poured into the dishes, but experience soon showed that the unsterilized solution remained free from contaminating fungi for a longer time than that which had been sterilized. This is due, possibly, to the hydrolysis of the sucrose during the sterilization process, the resulting monosaccharides offering more ready food to the contaminating fungi. Mudge (29), however, claims that practically no sucrose is inverted in an aqueous solution during sterilization. He sterilized solutions for as long as one hour under 15 pounds pressure and found practically no inversion. If this be true, the writer is at a loss to explain the uniform results obtained, unless they were due to the slight impurities in the commercial cane sugar used.

The ability of leaves and other portions of green plants to take up carbohydrates from solutions upon which they are floated has been observed by various investigators, among whom are Boehm (6), Meyer (27), Acton (1), Saposechnikoff (35, 36), Parkin (32), Atkins (5), Mains (21), and Tolenaar (44). Almost without exception they have found sucrose to be the most readily utilized of any of the sugars. They found that the optimum concentration range is from 4 to 20 per cent, depending on the conditions and type of leaf.

Among the nutrients tried during the present work were glucose, fructose, starch, maltose, raffinose, asparagin, malt extract, peptone, and cane sugar. In addition, Shive's (39) three-salt solution was added to various solutions, but the results showed that for all purposes of rust infection a simple solution of commercial cane sugar was the most satisfactory.

When leaves are kept in sugar solution, light is not necessary during storage, as the leaves absorb carbohydrates directly from the solution. Experiments, however, have shown that contamination by fungi is materially lowered when leaves in sugar solution are placed in diffused light instead of total darkness. At first they were kept under a black screen in the greenhouse, but during later experimental work they were kept on the same table as the leaves in the distilled water. In this way the leaves received the benefit of the carbohydrate solution plus any photosynthesis that may have been carried on.

The dishes containing the leaves upon distilled water had to be placed in a lighted place, as the rust-fungus obtains its nourishment only from the food which is stored in the leaf prior to inoculation or from the photosynthetic products formed in the leaf while in the dish. It was found, however, that if they were placed in direct sunlight the leaves were rapidly scalded. The plan finally used was to place them on a table in the north window where they received sufficient light to insure infection.

During the winter months when the days were short and the light in-

tensity low, electric lamps were suspended above the dishes. With the aid of these, infection was maintained satisfactorily.

The tables upon which the dishes were kept were washed thoroughly every other day with a two per cent solution of "Neko," a carbolic acid preparation (Parke Davis and Co.), to keep contamination at a minimum. The floor of the room was also washed with "Neko" occasionally. It was necessary to change the solutions in the dishes several times a week. The leaves were removed from the dishes, carefully washed in sterile distilled water, the dishes rinsed, and the leaves replaced in new solutions. If the petiole or the cut edges of the leaves appeared to be attacked by bacteria or molds, they were trimmed back so as to allow the continued entrance of the solution. The mortality rate with dish cultures is necessarily high because the leaf tissues are often bruised in handling. However, with careful observance of the rules of culture technique, a large percentage of the inoculated plants became infected. In practically all cases infection was more easily obtained on the leaves or portions of leaves in the petri dishes than on the natural host in the greenhouse.

Greenhouse Experiments with Potted Plants

The experiments given below have been grouped as far as possible according to the reactions of both host and parasite to certain environmental factors, such as light, temperature, moisture, and the resistance of the host. The influence of the food and chemical factors comes essentially from the host. As the temperature and light experiments were conducted at the same time, they will be grouped together.

Temperature and Light

The response of the host and parasite to these two environmental factors, both singly and combined, were investigated in nine rusts. They will be taken up in the order of experimentation.

Uromyces appendiculatus Fr., on *Phaseolus vulgaris* L. (variety: *Tennessee Green Pod*). In Experiment 1, twenty pots of bean were inoculated by spraying with a spore suspension and then placed in the moist chamber for 48 hours at a temperature of about 20° C. At the end of this time they were placed in the greenhouse and allowed to remain until urediniospores were formed. On the eighth day after inoculation, just as the uredinial pustules were breaking through the epidermis of the leaves, five pots were removed to a dark room at 19° C. and allowed to remain for two days. Five pots were also placed in a cold dark room at 7° C. and removed at the end of two days. The remaining ten pots were left in the greenhouse under ordinary conditions at 19–25° C. The results are shown in table 1.

TABLE 1.—*The effects of temperature and varying amounts of light on time of teliospore formation in Uromyces appendiculatus on Tennessee green pod beans*

Experiment 1

No. plants	Incubation period in days	Treatment after 8-day incubation period ^a	Formation of teliospores (no. days after inoculation)
5	8	In dark room, 19° C. for 2 days	17
4	8	In dark room, 7° C. for 2 days	15
9	8	Checks, in greenhouse at 20° C.	28

Experiment 2

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores (no. days after inoculation)
5	11	In dark room, 19° C. for 4 days	12
4	12	In dark room, 7° C. for 2 days	13
4	8	Checks, in greenhouse at 20° C.	26

Experiment 3

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores (no. days after inoculation)
3	11	In light, 7° C. for 2 days	13
3	11	In dark room, 7° C. for 1 day	13
4	12	In dark room, 19° C. for 4 days	13
4	8	Checks, in greenhouse at 20° C.	24

Experiment 4

No. plants	Incubation period in days	Treatment after 9-day incubation period ^a	Formation of telio- spores (no. days after inoculation)
5	9	In light, 7° C. for 2 days	19
4	9	In dark room, 7° C. for 2 days	17
4	9	In dark room, 19° C. for 2 days	24
5	9	Checks, in greenhouse at 20° C.	28

^a Urediniospores had formed at this time.

In explanation it may be said that under ordinary conditions of infection of bean leaves, secondary and tertiary circles of uredinia form about the primary sorus before the telial stage arises. When teliospores are formed, they usually appear in the two later-formed circles of pustules, seldom in the primary. In the above experiment, on those plants which were placed in the cold dark room, there was about 100 per cent teliospore production in the primary pustules at the end of the fifteenth day; on those which had been placed in the dark room at 19° C., there was 8 per cent teliospore production in the primary pustules; and in the case of the checks the teliospores were interspersed with the urediniospores in the secondary and tertiary pustules, with none in the primary. In the two former cases practically no secondary or tertiary pustules had formed.

These results show that both darkness and low temperature tend to suppress the uredinial stage and to favor the early production of teliospores.

In Experiment 2, the plants were removed to the dark rooms four days after inoculation to note the effect on the incubation time and also on time of teliospore formation. The results of this experiment are summarized in table 1, Experiment 2.

From the results of Experiment 2 it is seen that the incubation time is lengthened by approximately the time that the plants are kept in darkness and at low temperature.

That metabolism is not completely inhibited at 7° C. is shown by Experiment 3, in which the plants were placed in darkness at 19° C., in darkness at 7° C., and in a room at 7° C. which received light approximately 15 hours a day. In all these cases the plants were placed under the several conditions four days after inoculation. Results of Experiment 3 are summarized in table 1.

On all the plants with the exception of the checks, teliospores were found interspersed with urediniospores at the end of the incubation period.

It is apparent that light even at as low a temperature as 7° C. exerts a favorable, indirect effect upon the fungus, for the incubation period in this experiment was the same as that of the rust which was in the dark at 7° C. for only one-half the time.

In Experiment 4 the same conditions of temperature and light were used as in Experiment 3, except that the plants were placed under the changed environmental conditions at the time of the first appearance of uredinia, *i.e.*, nine days after inoculation. The results of this experiment show a relative delay in the formation of the spore forms. The incubation period was longer than usual, and the telial stage appeared later. However, the comparative results remained the same.

In the experiments thus far conducted, inoculations on the bean were always made upon the first two leaves that appeared, namely, the primordial leaves. As practically all the photosynthesis that takes place at this young stage of the plant is carried on in the primordial leaves, there is a constant withdrawal of a portion of such elaborated food for the development of the later foliage, the trifoliate leaves. By clipping back the later developing shoots it should be possible to retain a large part of this food in the primordial leaves. Thus if teliospore formation is an indication of a famished condition in the host plant, those plants that were clipped should show a comparatively longer period of urediniospore production than those that were allowed to maintain their normal growth. Accordingly, experiments were conducted to determine if this hypothesis were true.

In the next experiment, therefore, one-half of the inoculated plants were clipped and the other half allowed to retain the normal habit of growth. All plants were placed under the same environmental conditions. On those plants which had been infected and clipped, teliospores were produced from 32 to 34 days after inoculation, while on the unclipped plants the telial stage was produced from 26 to 28 days after inoculation.

The primordial leaves on the unclipped plants presented a decidedly different appearance than did those on the clipped plants. They were of a paler color and less leathery in texture, and at the time of teliospore formation showed the effects of the rust to a much greater degree than the others (Fig. 1). The sori upon the leaves of the unclipped plants were smaller, there were fewer secondary sori, and in general the leaves seemed to contain less food than those upon the plants which were clipped. The fact that the telial stage appeared on the unclipped plants from six to ten days earlier than on the clipped plants seems significant enough.

The comparative effects of temperature and light on the clipped and unclipped plants were determined by placing the plants in the several environments at the time of uredinial formation. The results are given in table 2.

TABLE 2.—*The effect of temperature and light on time of teliospore formation in Uromyces appendiculatus on "clipped" and "unclipped" plants of Tennessee Green Pod beans*

Plant no. and condition	Treatment after 7 to 8-day incubation period	Formation of telial stage	
		No. days after inoculation	Remarks
B 87-90 clipped ^a	In dark room, 7° C., for 2 days	14	In primary and secondary pustules.
B 91-93 unclipped	In dark room, 7° C., for 2 days	14-15	In primary pustules. No secondary ones found.
B 94-96 clipped	In dark room, 19° C., for 2 days	15-17	In secondary pustules.
B 97-100 unclipped	In dark room, 19° C., for 2 days	15-17	In primary pustules.
B 101-110 clipped	Checks, in greenhouse at 20° C.	30	In secondary pustules.
B 111-115 unclipped	Checks, in greenhouse at 20° C.	26	In secondary pustules.

^a Plants with growing point removed.

These results show that, when the plants are placed in darkness at either room temperature or reduced temperature, the formation of telia is not hastened appreciably by removal of the growing point. It is only when the plant is allowed to remain under normal conditions of light and temperature and when metabolism is going on at a normal rate, with a constant increase in size of the trifoliate leaves, that the drain on the food from the primordial leaves is made apparent by the earlier appearance of the teliospore generation.

Fromme and Wingard (13) assert that in resistant varieties of beans the incubation time may be lengthened and teliospores make their appearance without being preceded by the uredinial generation as is normally the case in susceptible varieties. These results were verified by the writer in experiments conducted under normal conditions and under conditions as outlined above. The Scarlet Wax bean was used as a resistant variety.

Plants of Scarlet Wax and Tennessee Green Pod of the same age were inoculated at the same time. Nine days after inoculation the uredinia broke through the epidermis of the susceptible variety, and three days later they appeared on the resistant one. Primary and secondary sori formed on both varieties but on the susceptible one the sori were larger, more numerous, and continued to produce urediniospores for a longer time than sori on the resistant one. Teliospores appeared on the Scarlet Wax 14 days after inoculation, but it was not until the twenty-third day that they

formed on the Tennessee Green Pod. In the former case they were produced in the primary and secondary sori, while in the latter case they were formed in the secondary only. On some of the plants of the Scarlet Wax variety, teliospores were found interspersed with urediniospores in the very first pustules. In order to note the effect of unfavorable conditions on such



FIG. 1. Rusted bean plants, showing condition of the primordial leaves on plants with and without the growing points removed.

a host during incubation, several plants of Scarlet Wax were inoculated and four days later were placed under different conditions of light and temperatures. Table 3 gives the results of this experiment.

From the results it is evident that, when resistant varieties of beans are infected with the rust, there is a tendency for the uredinial generation to be shortened or even suppressed. When plants are placed under unfavor-

TABLE 3.—*The effect of temperature and light on time of teliospore formation in Uromyces appendiculatus on Scarlet Wax beans*

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores	
			No. days after inoculation	Remarks
7	11	In dark room, 7° C., for 1 day	11	In primary pustules.
4	14	In dark room, 19° C., for 4 days	14	In primary pustules.
7	15	Checks, in greenhouse at 20° C.	15	In primary and secondary pustules.

able conditions during part of the incubation period, the uredinial generation may be eliminated entirely.

Puccinia suaveolens Rostr., on *Cirsium arvense* (L.) Scop. *Cirsium* rust, in nature, forms an abundance of teliospores throughout the growing season. Teliospores may be found on badly infected plants at all times, even in the primary systemic infections early in the summer.

Rostrup (34) and Olive (30) showed that *Puccinia suaveolens* was systemic and that the mycelium hibernates in the rhizome. Plants in the field infected with the primary stage of the rust never blossom, and they can be readily detected by their pale, spindly appearance. Adjacent plants are infected by the primary urediniospores which give rise to local infection, much less damaging in its effect.

For experimental material of this rust, plants were brought in from the field and planted in pots in the greenhouse. After becoming adapted to their new environment they were inoculated by spraying with a spore suspension, the spores of which were obtained from infected plants collected in the field. The incubation period of this rust varies from six to ten days, the optimum temperature for germination being about 18°–22° C.

The uredinia appear almost always on the upper surface of the leaf, although the primary uredinia usually appear on the lower. Later the sori may extend through to both surfaces. Under optimum conditions of temperature and moisture the rust may not produce teliospores for more than a month after infection. In most cases, however, the uredinia are found to contain teliospores as early as two weeks after infection. The teliospores appear on the lower leaves first and then extend progressively up the plant. This rust will not propagate itself in the greenhouse but must be re-inoculated about every 30 days in order to keep it in the uredinial stage. Under conditions of high temperature and low humidity the regions surrounding the uredinia will become dry and the rust will die.

The first experiment with this rust was conducted in order to note the effects of temperature and light on the type of spore that would be produced.

Twenty potted plants were inoculated and placed under various conditions of light and temperature similar to those used in the experiments with the bean. The results are shown in table 4.

TABLE 4.—*The effect of temperature and light on the time of teliospore formation in Puccinia suaveolens on Cirsium arvense*

No. plants	Incubation period in days	Treatment after 7-day incubation period	Formation of teliospores (no. days after inoculation)
5	7	In dark room, 19° C., 5 days	28
6	7	In dark room, 7° C., 5 days	24
10	7	Checks, in greenhouse at 20° C.	32

These results show that telial formation can be hastened by subjecting the infected plant to conditions unfavorable for metabolism in the host. When the plants were removed from the dark rooms, the areas surrounding the pustules were lighter than the remainder of the leaves and on some of the plants these areas were completely dried up.

In the next experiment an attempt was made to induce the formation of teliospores by removing the plants from pots, immersing their roots in distilled water, and placing them in the dark at a temperature of 19° C. Plants C20-25 and C25-30 were removed from the pots of earth at the time of uredinial formation and placed in flasks of distilled water, the roots being carefully washed of all particles of soil. Here they remained for two days in the sunlight, after which they were placed in the dark room for two days. At the end of this time plants C20-25 were placed in flasks of Shive's nutrient solution and returned to the sunlight, while plants C25-30 were left in distilled water and returned to the sunlight. Plants C30-35 and C35-40 were left in pots during the entire experiment (Table 5).

On plants C20-25, which were transferred at the time of uredinial formation to distilled water for two days, and then placed in the dark room for two days and finally transferred to Shive's solution for the remainder of the time, teliospores appeared on the sixteenth day after inoculation.

On plants C25-30, which were placed in distilled water at the same time as C20-25, allowed to remain for two days, and transferred to the dark

TABLE 5.—*The effect of light and nutrient solution on teliospore formation in Puccinia suaveolens on Cirsium arvense*

Plant no.	Treatment after 6-day incubation period	Formation of teliospores	
		No. of days after inoculation	Remarks
C20-25	Removed from pots and placed in distilled H ₂ O in light for 2 days, then in darkness for 2 days; transferred to Shive's solution and placed in light	16	Teliospores numerous.
C25-30	Do ; left in distilled H ₂ O, not Shive's solution	—	Pustules dried up. No teliospores.
C30-35	Potted plants in dark for 2 days, then returned to light	25	Teliospores numerous.
C35-40	Checks, in greenhouse	32	Teliospores mostly on lower leaves.

room for two days, there was no telial formation whatever. The portions of the leaves around the rust pustules dried up and a few days after removal from the dark room the rust had apparently died. The remaining plants, C30-35 and C35-40, were accorded the same treatment as plants in the earlier experiment, not being transferred from pots, and the results were practically the same.

Plants C20-25 continued to produce new leaves in the Shive's solution for 60 days after the transfer, the rusted leaves slowly drying up and dropping off, leaving the plants free from rust.

Results of other experiments similar to these were the same; in all cases the placing of infected plants in the dark either at low or room temperature was sufficient to inhibit further formation of urediniospores. In some cases, if the plants were not allowed to remain under such conditions for too long a period, uredinial production was resumed in the same pustules after teliospores had been produced. Especially noticeable was this in *Uromyces polygoni*, *Puccinia antirrhini*, and *Puccinia taraxaci*.

During winter days when the light intensity was low and the hours of daylight few, it was very difficult to maintain a stock supply of urediniospores of *Puccinia suaveolens*. When plants were infected, in many cases the first pustules that broke through contained 50 per cent of teliospores. In no case was it possible to maintain the uredinial stage for more than two weeks after the first infection. This difficulty was partially overcome by installing seven 100-watt electric lamps in the experimental room of the greenhouse. These were suspended about three feet above the pots and were supplied with reflectors to concentrate the rays of light. With the

aid of this auxiliary illumination, which was turned on from about four o'clock in the afternoon until eleven at night, it was possible to secure infection and prolong the uredinial stage to three weeks or more.

Uromyces trifolii Lev. on *Trifolium hybridum* L. Plants of alsike were grown from seed and inoculations made in the usual manner. The incubation time of this rust varies from 7 to 10 days, the optimum temperature for spore germination being about 18–20° C. The pustules are first evident as brownish areas on the under side of the leaves, the uredinia first breaking through on that surface, then later extending through to both surfaces. Under ordinary conditions in the greenhouse, teliospores are not formed in appreciable quantities, the leaves usually drying up before such a stage is reached. Occasionally they are formed on the petioles of the older infected leaves, and in very rare cases upon the blades, interspersed with urediniospores. This condition of course will vary, depending upon the amount of infection and environmental conditions.

The first experiment with this rust was to determine the effects of light and temperature upon the type of spore that would be produced.

Young plants, varying from four to five inches in height, were inoculated. At the time of appearance of the uredinia, some were placed in the dark at a temperature of 19° C., and others in the dark at 7° C. The results are shown in table 6.

TABLE 6.—*The effect of light and temperature on time of teliospore formation in Uromyces trifolii on Trifolium hybridum* L.

No. plants	Treatment after 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 7° C., for 4 days.	—	No teliospores formed. Leaves dried up.
4	In dark room, 19° C., for 4 days.	32	Teliospores on petioles only. Few in number.
6	Checks, in greenhouse at 20° C.	46	A few scattered teliospores on petioles.

It is evident that the treatment accorded the plants placed in the cold dark room was too severe to allow the production of any additional spores after the plants were removed. The leaves had completely withered and the plants had not grown after being placed in the dark. The host was too rapidly weakened to support the formation of teliospores. Even in the case of those plants placed in the dark at room temperature, the leaf blades had withered and the few teliospores that had formed were found upon the peti-

oles of the leaves. It was evident from these experiments that some method less drastic must be devised which would extend over a longer period of time in order to avoid killing the host too soon.

In the next experiment the plants were placed under the changed environmental conditions for shorter periods of time (Table 7).

TABLE 7.—*The effect of varying periods of light and temperature on time of teliospore formation in Uromyces trifolii on Trifolium hybridum*

No. plants	Treatment after 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 7° C., for 1 day; in dark room, 19° C., for 1 day; in greenhouse at 20° C. for 3 days; in dark room 7° C., for 1 day.	36	Some telia on leaf blades but mostly on petioles.
2	In dark room, 7° C., for 2 days.	35	Teliospores on petioles.
3	In dark room, 7° C., for 3 days.	29	Few teliospores formed. Leaves badly injured.
2	In dark room, 7° C., for 4 days.	28	Very few teliospores, on petioles. Infected leaf blades dead.
2	In dark room, 7° C., for 6 days.	—	Leaves killed. No teliospores formed.
8	Cheeks, in greenhouse at 20° C.	45	A few teliospores on petioles. Leaf blades dead.

In the first set of plants the gradual decrease in metabolism of the host due to the different environments was sufficient to stimulate the formation of the telial stage. That the host was not seriously injured by the treatment was evidenced by the fact that telia were formed on the blades of some of the leaves. In a similar manner the telial stage was produced on the plants which had been in the cold dark room for a period of two days. In this case, however, the blades showed the effects of the darkness and low temperature and had completely withered. The same effects were apparent in the plants which were placed therein for longer periods of time. It is evident that on this host it is difficult to reach the exact condition wherein the uredinial stage will be inhibited and the telial stage produced without killing both the host and the parasite.

As it has been shown by Acton (1), Boehm (6), and others that roots, stems, and leaves will take up carbon compounds from solution, whole plants of alsike were placed in solutions of sucrose and submitted to varying conditions of light in an attempt to lower the metabolism of the host to a point that would inhibit uredinial production and at the same time not cause the death of the host.

Sixteen plants were inoculated. At the time of uredinial formation, 12 of them were removed from the pots, the roots thoroughly rinsed and cleared of soil particles, and placed in 100 cc. flasks of seven per cent sucrose solution. The remaining four plants were allowed to remain in the pots as checks. Eight of the plants in the sucrose solution were placed in the dark room at a temperature of 19° C. and the remaining four left in the greenhouse with the checks.

The solutions were changed every day and the roots of the plants rinsed in a one per cent solution of Zonite (a commercial preparation of NaOCl held in electrolyzed hypertonic solution), after which they were thoroughly rinsed in distilled water and replaced in the new sucrose solution. In this way contamination was kept very low, and the roots did not seem to be injured by the application of Zonite.

Two days later, four of the plants in the dark room were transferred from the sucrose solution to distilled water for four days, after which they were again placed in the sucrose solution. It was noticed at this time that the petioles of the plants in the dark room contained large numbers of uredinia, while those in the greenhouse in the light had only about one-half as many.

At the end of the tenth day after inoculation, teliospores were observed on the leaf petioles of those plants which had been transferred from the sucrose solution to distilled water and back again. Three days later they were also found on the plants which had been in the dark continuously on the sucrose solution. They were far more numerous on the latter plants, each pustule containing 50 per cent teliospores. On the former the teliospores were few and interspersed with urediniospores in only about 30 per cent of the pustules. Three days later teliospores appeared on the plants in sucrose in the greenhouse, and on the potted plants in the greenhouse on the thirty-eighth day following inoculation.

In practically all cases in this experiment teliospores were formed on the petioles and not on the leaf blades. The leaves of the plants in the dark withered soon after they were placed there, the ones on distilled water dying before the others. It appears that the plants which were on the sugar solution continuously in the dark, although they did not produce the telial stage so soon as those which were transferred to water, absorbed more of the nutrient from the solution and as a result produced more teliospores than

the other plants. The plants on water felt the hunger stimulus first but did not have the food supply to produce the abundant crop as did the others.

Puccinia taraxaci Plowr. on *Taraxacum officinale* Weber. *Taraxacum* rust is similar to *Puccinia suaveolens* in that it produces pycnia accompanied by primary uredinia instead of by aecia. Infections by primary urediniospores produce the regular secondary urediniospores followed by the telial stage. The incubation period of *P. taraxaci* varies from 8 to 12 days; the uredinia first appear on the lower surface, and usually break through the upper epidermis later. The optimum temperature for spore germination appears to be about 18–20° C.

The telial stage of this rust is seldom found in the greenhouse and, according to the experience of the writer, has never been seen there except under experimental conditions as described below. There appears to be great variability in the resistance and susceptibility of host plants to this rust, for plants have been brought into the greenhouse which have never become infected as a result of inoculation, either in pots or petri dishes. Other individuals transferred from the same vicinity in the field showed great susceptibility under both conditions. In the work with this rust, as well as with the others, care has been taken to limit the experimental work to one strain of rust, so that, to the best of the writer's knowledge and belief, discrepancies from this source have been kept out. Any difference in infection ability has been due to the host alone.

Various experiments were tried with light and temperature, similar to those already reported, but in no case under these ordinary methods was the telial stage produced. An infected plant may continue to produce urediniospores until the leaf is entirely covered with pustules. Placing the plants in darkness and under low temperature may cause a temporary lessening of uredinial production, but shortly after the plants are again placed in a favorable situation copious uredinial production is resumed.

Infected plants were placed in the dark at a temperature of 19° C. and also in the dark at 7° C. and left for periods varying from one to seven days, but in no case were teliospores produced. In extreme cases the host was killed without ever showing any signs of the resistant spore. In all, 65 infected plants were used, and not a single teliospore was produced.

Finally an attempt was made to vary the conditions in such a way as slowly to bring the metabolism of the host plant to a condition similar to that existing in nature during the late growing season when the telial stage is produced.

Taraxacum plants were inoculated. Three days after the first appearance of uredinia, plants T10–15 were placed in the cold dark room at 7° C.

for three days. They were then transferred to the greenhouse for seven days and again removed to the cold dark room for six days. Five days after they had been returned to the greenhouse, teliospores appeared in the old uredinial pustules. They were not numerous, averaging about one teliospore to every ten urediniospores. They continued to form for four days, until, under the influence of the greenhouse environment, urediniospores were again produced exclusively. The plants were then transferred to the cold dark room for three days and, three days after their removal to the greenhouse, urediniospore production ceased and teliospores were again formed in the old pustules. This continued for several days to the exclusion of the urediniospores, until finally the infected leaves died, leaving the plant rust-free.

This experiment shows that it is possible to induce telial production even in those plants which do not produce the telial generation readily under the ordinary experimental methods used for some of the other rusts. The principle, however, is the same in this case as in the others.

Puccinia antirrhini Diet & Holw., on *Antirrhinum majus* L. Plants of *Antirrhinum* were raised from seed in the greenhouse and inoculations made in the usual manner. The incubation period of *Puccinia antirrhini* ranges from 7 to 14 days. The optimum temperature for spore germination is about 19-20° C. A temperature of from 25 to 30° C. will gradually free the host of the parasite.

The first evidence of infection on the host plants are light areas on the under surfaces of the leaves. These gradually give way to the uredinia, which seldom extend through to the upper surface. The uredinia are large and circular, and under optimum conditions increase in a radial direction by forming concentric rings of uredinia outside of the original sorus. The uredinial and telial generations are the only ones that have been observed on *Antirrhinum*, and, until Hackey (15) and Mains (23) succeeded in germinating teliospores, they were generally thought to be functionless.

Under ordinary greenhouse conditions, teliospores are seldom produced on actively growing plants. Doran (10) succeeded in producing them on plants which had been gradually deprived of water. This probably accounts for their presence occasionally on old stems of infected plants which have lost their leaves in the greenhouse.

Doran (10) reports finding teliospores on plants outdoors after the low temperature of autumn had set in. The writer has found the telial stage both on the leaves and stems in the field in November, although telia were scarce on the leaves.

The first attempt to inhibit the continued production of urediniospores and so obtain formation of teliospores was made in the same manner as for

the earlier mentioned rusts. Plants were placed in the cold dark room immediately on the appearance of uredinia and allowed to remain at 7° C. for periods of time ranging from 2 to 6 days. On being returned to the greenhouse, the urediniospores continued to form on those leaves which had not been seriously injured by the low temperature. By varying the time left in the dark and allowing the plants partially to recuperate in the light after each exposure to low temperature, teliospores soon made their appearance. The results are given in table 8.

TABLE 8.—*The effect of light and temperature on the time of teliospore formation in Puccinia antirrhini on Antirrhinum majus*

No. plants	Treatment after 10-day incubation period	Formation of teliospores (no. days after inoculation)
4	In dark at 7° C. for 3 days; in light at 20° C. for 10 days; in dark at 7° C. for 3 days	21
5	In dark at 7° C. for 4 days	No teliospores.
5	In dark at 7° C. for 6 days	do
8	Checks, in greenhouse at 20° C.	do

The telial stage appeared only on those plants placed at low temperature in darkness at two different times, with an interval between; this permitted the host to withstand the effects of the unfavorable environment without serious injury. On the plants which were subjected to the same treatment only once, there were no teliospores. It would appear that, similar to the case of the Taraxacum rust, it required a more extended and gradual treatment to upset sufficiently the metabolism of both the host and the parasite so that the latter was stimulated to produce teliospores. The tips of the infected leaves on all of the plants were withered, and it was only back of these withered areas that teliospores were produced. They were in most cases interspersed with urediniospores, but on a few of the leaves separate telia were formed near the withered tips. It appeared as if the mycelium of the parasite were spreading from the generally infected area back into the uninfected regions of the leaf and there encountering the conditions which stimulated it to produce teliospores. The region of the leaf surrounding the telia was not withered and did not have the appearance of being seriously injured. The regions around the uredinia were severely injured, and it appeared as if these parts of the leaf had succumbed so rapidly that the fungus did not have time to produce the resistant spores.

In the next attempt to produce teliospores, eight plants were transferred, at the time of urediniospore formation, from pots of earth to flasks of distilled water, the roots only being submerged. The flasks were placed in the

dark room at a temperature of 22° C. for three days. At the end of this period the plants were repotted and returned to the greenhouse. Ten days later teliospores appeared in the same manner as before; most of them were interspersed with urediniospores, but a few were in separate telia. They did not continue to form for any length of time, and the same sori, three days later, disclosed the formation of new urediniospores on the outer edges of the old pustules. Thus it appears that, after the stimulus which had induced the formation of teliospores had been removed, the same mycelium was capable of resuming the production of the uredinial generation.

Uromyces polygoni Fuckel, on *Polygonum aviculare* L. Rusted plants of *Polygonum aviculare* were brought in from the field near Ann Arbor and planted in pots in the greenhouse. Uninfected plants from this general region were also placed in pots and inoculated with material obtained from the infected hosts.

The incubation period of *Uromyces polygoni* varies from 8 to 15 days, the uredinial sori first breaking through the leaves on the lower surface and later extending through to the upper. This rust is exceedingly prolific and will spread over an entire plant in a short time even in the greenhouse. The telial stage forms very readily and is produced upon old leaves of infected plants during the entire growing season.

Infected plants P1-5, on the first appearance of the uredinial stage, were placed in the dark room at 7° C. for four days, afterwards being removed to the greenhouse with the checks. Plants P5-10 were placed in the dark room at 19° C., at the same time for the same length of time.

Four days later teliospores were found on plants P1-5 and plants P5-10. In the case of the former, the production of teliospores was almost 100 per cent on all of the infected leaves. They were produced in the old uredinial pustules and were evident only after the old urediniospores had been scraped away. On plants P5-10 they were not so numerous and were only formed on those leaves which were older and more heavily infected. On the checks teliospores were not yet being produced except on some of the lower, older leaves which had been severely injured by the rust.

In four days urediniospores could be found again on all of the plants, the majority being produced around the outer margins of the old pustules. The plants were again subjected to the same conditions as before, and three days after removal to the greenhouse teliospores were being formed for the second time. They were produced on plants P1-5 until the infected leaves dropped and left the plant rust-free. On plants P5-10 teliospores were produced for several days, then urediniospores were formed again for a short time before the rust finally died. On the check plants, meanwhile,

urediniospores were produced, except on the older infected leaves, for several weeks, until gradually the rust died.

Puccinia triticina Erikss., on *Triticum vulgare* Vill. Plants of the Golden Wave variety of wheat were grown from seed in pots in the greenhouse and infected with material of *Puccinia triticina* received from Dr. E. B. Mains, of the Purdue Agricultural Experiment Station, Lafayette, Indiana. The incubation period varies from 6 to 12 days. The first signs of infection are light-colored areas on the leaves, through which the uredinia soon break. Leaves infected with *P. triticina* soon dry up, and unless they are reinoculated about every 15 days it is impossible to maintain the rust in the greenhouse.

In all of the experiments made with this rust in the greenhouse, the writer was not able to produce a condition of the host whereby the uredinial stage was inhibited and the telial stage stimulated, although such a condition is not to be considered impossible of attainment in greenhouse experimentation.

Rusted plants were subjected to darkness, low temperatures, dessication, etc., but in no case were teliospores produced. They were, however, induced on detached leaves in petri dishes. A description is given later.

Puccinia sorghi Schw., on *Zea mays* L. The effects of light and temperature upon the production of teliospores by *Puccinia sorghi* was demonstrated by several experiments similar to those conducted for rusts so far mentioned.

Mains (21) carried on exhaustive cultural experiments with this organism and gives an account of its reactions, so that further discussion of the preliminary procedure is unnecessary.

Plants were inoculated in the usual manner, and at the time of first uredinial formation were put in darkness and at low temperatures. The results are shown in table 9.

In this situation we find a condition somewhat similar to that present in the wheat. It is difficult to adjust the conditions for control of the metabolic processes in the host in such a manner that the rust will be able to respond without first killing both the host and its parasite. On only one of the four plants placed in the dark room at 19° C. were teliospores produced, and on two of the four placed in the dark room at 7° C. In both of these cases they were very scattered and were interspersed with urediniospores in the center of the uredinia. In the other plants either the infected leaves died, or the region surrounding the sorus appeared to be completely dried out, leaving the rust mycelium isolated from the food in other regions of the leaf. It is interesting to note that in infected leaves exposed to light the areas surrounding the sori retain their green color long

TABLE 9.—*The effect of light and temperature on time of teliospore formation in Puccinia sorghi on Zea mays*

No. plants	Treatment after 6 to 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 19° C., for 4 days	—	Leaves dried up. No teliospores formed.
6	In dark room, 19° C., for 3 days	—	do
4	In dark room, 19° C., for 2 days	20	A few teliospores formed in with the urediniospores on one of the plants.
5	In dark room, 7° C., for 3 days	—	Leaves dried up. No teliospores formed.
4	In dark room, 7° C., for 1 day	17	Teliospores formed on two of the four plants in the old uredinial pustules.
10	Checks, in greenhouse at 20° C.	—	No teliospores formed. The older leaves gradually dried up and left the plant rust-free.

after the other portions have faded, while the reverse is true of plants placed in the dark.

This phenomenon has been observed by Ward (48), Tubeuf (45), and Mains (21) in their experiments with rusts.

Puccinia asparagi DC., on *Asparagus officinale* L. Seeds of asparagus were planted in pots in the greenhouse, and when the plants were about eight inches tall they were inoculated with *Puccinia asparagi* collected in the field near Ann Arbor.

The incubation period of this rust is quite variable, depending largely on the age and condition of the host. Very small plants are not easily infected. In several of the inoculations the time was from 12 to 18 days. In petri-dish culture, to be taken up later, the incubation time ranged from 12 to 20 days.

In all the experiments on the effect of temperature and light the results were negative as far as production of teliospores was concerned. Infected plants were subjected to various conditions but in all cases died before any results could be expected.

Moisture Relations of Teliospore Production

The moisture relation of *Puccinia asparagi* can, according to Smith (40), be considered under two heads; the direct effect of atmospheric moisture on the spores or mycelium of the fungus, and the indirect effect of moisture on the parasite through its effects upon the host. The latter, of course, means soil moisture.

The writer has attempted to verify by experimental methods in the greenhouse the field observations of Smith upon the moisture relations of *P. asparagi*.

Potted plants of asparagus were inoculated with the uredinial stage of the rust and the infected plants apportioned for the experiments as follows:

Two of the plants, A1-2, were placed in the greenhouse where the humidity was relatively high and given only enough water to keep them alive. Two, A3-4, were placed under bell-jars in a well-lighted place with the pots standing in water. The remaining two, A5-6, were placed in the greenhouse by the side of A1-2 and kept well watered.

One week later, on plants A1-2 teliospores appeared in the center of the old uredinial pustules. In another week the entire infected regions of the stalks were filled with teliospores and the fungus had ceased to spread.

On plants A3-4 the rust continued to form urediniospores for two weeks, new sori appearing as concentric circles around the old. The urediniospores were heaped up in the sori with exceedingly long pedicels, and behaved as the rust did when cultivated in petri dishes on detached stalks of asparagus. This is probably due to high humidity, since Hennings (16) observed the same conditions on rusted plants in very humid environments. During the third week after infection teliospores appeared in the center of the first formed uredinia and gradually spread outward but with much less rapidity than in plants A1-2. By the end of the fourth week the pustules were exclusively telial and the infected stalks were killed.

On plants A5-6 teliospores were produced at the end of the second week in the center of the first formed uredinia. The former spread outward in a radial direction preceded by the circles of uredinia. By the end of the third week the sori were completely made up of teliospores and the stalks were dead.

We thus get different results under three sets of different environmental conditions: first, high humidity and high soil moisture; second, moderate humidity and high soil moisture; third, moderate humidity and low soil moisture.

Under the first set of conditions, prolific urediniospore production occurred for nearly three weeks before the telial stage appeared, and even then the new uredinia continued to form for almost two more weeks before teliospores were produced in all of the sori.

In the second case teliospores appeared during the second week, preceded by uredinial production until the end of the third week.

In the last situation the telial stage appeared during the first part of the second week, and before another week had passed the pustules were completely telial in nature and the spread of the fungus had ceased.

It is evident that soil moisture played an important rôle in determining the type of spore produced, for plants A1-2 and A5-6 were exposed to precisely the same humidity, but the amounts of soil moisture differed. That humidity played a part is also evident, as plants A3-4 and A5-6 had practically the same amounts of soil moisture but different humidities.

Four more potted asparagus plants were inoculated and, at the time of uredinial formation, two of them, A12-13, were placed in the south window of the laboratory room, while the other two, A14-15, remained in the greenhouse. Both sets of plants received plenty of soil moisture, practically the only difference in environment being the relative amounts of humidity.

Plants A14-15 developed in the same manner as did A1-2 in the previous experiment, the uredinial sori spreading radially, followed by the production of teliospores in the old pustules. By the end of three weeks spore production had almost ceased, teliospores occupying the whole of the sori.

Plants A12-13 formed teliospores at the end of seven days in the primary and secondary sori previously occupied by urediniospores. No uredinia formed beyond the second ring, and by the end of two weeks the spread of the rust had been checked. The plants were then taken from the laboratory and placed under bell-jars in the greenhouse with plenty of soil moisture in the pots. Six days later new uredinia were being formed exterior to the old sori, followed shortly by teliospores.

The results of the latter experiment added to those of the previous one make it certain that moisture, both in the air and soil, plays an important part in the type of spore produced by the fungus. However, can we say that either has a direct effect upon the fungus parasite? Until we know exactly what influence the shifting of environment had upon both the host plant and the parasite, we are not justified in ascribing to moisture of any kind a direct influence upon the fungus alone. Smith (40) says that when green stalks containing telial material are placed in moist chambers "there immediately breaks out at the outer edge of the infected area a circle of uredosori, with spores capable of immediate germination." This he ascribes to the direct effect of atmospheric moisture upon the rust fungus, without considering that back of the fungus is the living host with its complex of metabolic processes. Did the placing of the stalk in the moist chamber change the metabolic activities of the parasite and leave completely unchanged the cells of the living substratum upon which the fungus was growing? Certainly, from a physiological standpoint, the host in the moist

chamber was a different host than the one on which the teliospores were being produced. To the writer, it appears that, until we can separate the rust parasite from its obligate host and culture it upon an artificial medium, we cannot be justified in assuming that a change in environment of any kind will directly affect the fungus without at the same time having an equal effect upon the cells of the living host. These, in turn, through their change in metabolic activity, will exert a far greater influence upon the obligate parasite than any external factor such as moisture, light, temperature, etc.

Inasmuch as we can safely assert that the living host cells are the sole means of food supply for the rust fungus, and as the increased activity of such cells in a congenial host increases the activity of the parasite, any influence that is favorable for the host cells will be correspondingly favorable for the fungus. Thus when asparagus plants were brought from an environment of low moisture to a more favorable one of high moisture, metabolism increased—more food was made available for the rust—with the result that the uredinial generation was again produced.

In the case of bean rust the moisture relations, while not so marked as in the asparagus, nevertheless were noticeable. On rusted plants slowly deprived of water the telial stage appeared earlier than on plants which were kept well supplied.

Infected bean plants were divided into two lots, one brought into the laboratory at the time of uredinial formation and placed in an east window, the other allowed to remain in the greenhouse. Both were kept well supplied with soil moisture.

Three weeks from the time of inoculation, on the plants in the laboratory, teliospores were forming in the old primary uredinia, while none appeared on those in the greenhouse for another week. In the latter case secondary and tertiary uredinia had been formed, and it was in the tertiary pustules that teliospores appeared. While the light factor may have entered into the experiment to some extent, it was not sufficient to account for the extreme contrast in results. Repeated experiments produced the same results; even when additional light was supplied to the plants in the laboratory by means of electric lamps, the teliospores always appeared from five to seven days sooner than on the plants in the greenhouse.

In the case of snapdragon rust, Doran (10) has already shown the effect of moisture on production of teliospores. In the experiments conducted to repeat those of Doran, the teliospores always made their appearance on the stems and never on the leaves. Infected plants, with uredinial lesions on both the leaves and stems, were gradually deprived of water, and in four weeks after the treatment was begun teliospores were produced on the stems. The leaves meanwhile had dried up, only the uredinial generation

having been produced on them. Those plants which were kept well supplied with water were always killed by the rust before the telial stage was reached.

Petri-Dish Experiments

Eleven rusts, including the nine already mentioned, have been cultured on leaves or portions of leaves floated on water or aqueous solutions in petri dishes. Both urediniospores and teliospores have been produced in eight of these rusts, the type of spore depending in each case on the treatment to which the leaves of each host were subjected. In all the experiments recorded, a five or seven per cent cane sugar solution was used, the former being employed only in the first experiments with bean rust.

Uromyces appendiculatus Fr., on *Phaseolus vulgaris* L. Bean rust is exceedingly easy to culture in dishes and forms its telial stage very readily when placed under conditions that are unfavorable for the host. It is a mistaken notion to think that a weakening of the host predisposes it to the ravages of a rust. Quite the contrary is true. In order to insure a well developed growth of a rust it is quite essential that the host, presuming it to be a susceptible one, be in a healthy vigorous condition (Arthur, 3, and Stakman, 41). If the host is weakened or stunted, the rust either will not develop or will be weakened and produce small pustules and pale spores, and in some cases the uredinial stage will be shortened and the telial stage produced prematurely. A fairly vigorous condition of leaves can be brought about by the use of nutritive sugar solutions; hence it is possible to use the petri dish method for a study of rust reactions.

A few preliminary experiments with bean rust have already been reported upon by the writer (50), and these have shown the ease with which the spore stages can be controlled. During the first experiments it was noted that when bean leaves on distilled water were inoculated, the uredinial pustules were always smaller and paler than when the leaves had been floated on a sugar solution. On leaves on distilled water the pustules seldom increased in number as they did under favorable conditions, as when leaves were on sugar solutions. When teliospores were formed on the leaves on water they usually appeared in the primary pustules, and the rust died in from five to seven days after the first appearance of the uredinial stage. On the other hand, when the leaves were on sugar, secondary and tertiary pustules were usually formed, and when teliospores did make their appearance they were nearly always formed in the two latter sori, seldom in the primary. Quite commonly the rust became so active that the leaf was killed without ever forming teliospores. Frequently the leaf was a mass of uredinia with the spores heaped up to a height of a millimeter or more in the sori. Under such conditions telia were seldom formed.

In all of the dish experiments, the amount of infection played an important part in the appearance or non-appearance of teliospores. A light infection with few sori usually meant a long continuance of the uredinal stage if the leaf was kept on the sugar solution; changing such leaves from sugar to distilled water usually hastened somewhat the appearance of the telial stage. Frequently the reverse was also true. An infected leaf which had been on distilled water was likely to produce the telial stage when suddenly transferred to sugar solution.

Young primordial leaves were taken from healthy bean plants and placed in dishes containing a small amount of distilled water. They were sprayed with a spore suspension and set aside for 48 hours. At the end of this time a seven per cent sucrose solution was poured into one-half of the dishes in sufficient amounts to float the leaves freely. The petioles were clipped and the ends immersed to facilitate the entrance of the nutrient. At the time when uredinal pustules were just appearing on the leaves on sucrose, one-half of them were rinsed thoroughly and placed on distilled water. Likewise one-half of those on distilled water were placed on the sugar solution. The leaves were then set aside for seven days, at the end of which time they were examined.

The following scheme will be followed for designating the various treatments given the leaves in dishes:

A—Leaves which had been continuously on distilled water.

B—Leaves which had been on sucrose continuously.

AA—Leaves which had been transferred from sucrose to distilled water.

BB—Leaves which had been transferred from distilled water to sugar.

A. A fair amount of infection was obtained, but the sori were small and pale in color. The leaves were in a good state of preservation and did not seem to be damaged to any great extent as a result of the rust. In the pustules a few teliospores were interspersed with urediniospores, the latter predominating.

B. Heavy infection was secured, with an abundance of secondary uredinia. The leaves were considerably faded with the exception of the regions immediately surrounding the sori. No teliospores were found.

AA. Infection was almost as heavy as in B, but there were fewer secondary uredinia. Teliospores were abundant in the secondary pustules.

BB. The heaviest infection of all was obtained. There were not so many secondary uredinia, but the primary sori were more numerous and larger than those on the other plants.

One week later a second examination of the dishes was made, with the following results:

A. Growth was very scanty; the sori were very small; and comparatively few secondary pustules had formed. The sori, both primary and secondary, except on those leaves which had unusually heavy infections, contained teliospores almost entirely. The heavily infected leaves had no teliospores; they were in an impoverished condition, and the rust appeared to be famished to a point where it no longer had the ability to form teliospores. Leaves on which teliospores had formed and on which the infection was only moderate, were still green and did not appear to be injured by the parasite.

B. Many of the leaves were yellow and dead. The secondary and tertiary pustules contained an abundance of teliospores. As in A, in cases of very heavy infection, no teliospores were formed, and from the appearance of the leaves it seemed reasonable to suppose that the fungus developed with such intensity and speed that the cells of the host were killed and the fungus was practically isolated from the food supply and perished without the time or ability to form the resistant spore.

AA. There was practically no change in the development of the parasite. The leaves were almost entirely yellowed, and the production of teliospores in the primary and secondary sori had not increased much since the last examination. It would appear that, when water replaced the sugar solution, the fungus gradually stopped growing and producing teliospores.

BB. There was further deterioration of the leaves with not much change in the development of the fungus except that teliospores had formed in the secondary and tertiary sori. The areas immediately surrounding the sori were still green in color but did not appear to be alive. It appeared that this area had been isolated from the surrounding tissue and had dried up without losing its color.

The appearance and condition of the leaves of the bean, both in dishes and when attached to the plant, at the time of teliospore formation, suggested a test for the amount of stored food in the leaf at such a period. As the bean leaves store large amounts of transitory starch and practically no sugar, it seemed reasonable to suppose that, even though the rust existed upon some transitory products of photosynthesis or even upon food of a protein nature, the presence or absence of starch in the leaf would give very good indications of the food situation in the leaf. Accordingly, starch tests were made on leaves from the host plant and in petri dishes at different stages in the development of the rust, *i.e.*, when producing urediniospores and also when forming teliospores. The evident correlation between the amount of starch in the leaf and the type of spore produced at that time seemed to be strong proof that the appearance of teliospores on bean leaves indicated a paucity of carbohydrates in the host cells.

The results of these tests follow:

1. Leaves from plants in the greenhouse tested two days after the first appearance of the uredinial stage had large amounts of starch throughout the leaf. (Leaves for all tests were gathered at four o'clock in the afternoon, so that in these comparative tests translocation had not progressed to any great extent.)

2. In leaves from greenhouse plants tested 10 days after the appearance of the uredinial stage, the starch areas were localized around the uredinia. The areas distant from the fruiting pustules showed little or no starch.

3. Tests made of leaves 28 days after inoculation and bearing teliospores gave almost no test for starch. Even the areas surrounding the pustules gave only a faint starch reaction.

In plants which had been clipped, the reaction for starch was evident for longer periods than on those plants which had retained their trifoliate leaves. In general, the amount of starch in the leaf varied inversely with the number of teliospores.

Tests made on leaves which had been floated on sucrose solutions and distilled water in petri dishes showed the same variation:

1. Lightly infected leaves on distilled water showed a fair starch reaction at the time of uredinial formation.

2. Leaves on distilled water with heavy infections showed little or no starch at the time of uredinial formation. Such leaves usually had no teliospores.

3. Infected leaves on seven per cent sucrose solution gave an extremely strong starch reaction at the time of uredinial formation, the starch being denser along the cut edges of the leaves and along the larger veins.

4. Infected leaves on sugar at the time of telial formation had little or no starch. In cases of heavy infection where no teliospores were formed, there was practically no starch.

Attempts were made to show the effects of low temperatures upon the formation of teliospores similar to experiments made upon the plants in the greenhouse, but the results were negative.

It was found, on bringing the leaves from low to high temperatures, that rapid decomposition set in, which made it impossible to work with them. Another reason for the inability to perform temperature experiments with detached leaves is the fact that at low temperatures very little starch is formed in the leaves and the development of the rust is at a standstill. The reaction of leaves and their storage of starch at low temperatures has been investigated by Czapek (9), Lidforss (19), and Tolenaar (44), who found that starch storage took place at low temperatures to a much less degree than at high.

Uromyces trifolii Lev., on *Trifolium hybridum* L. Infection of alsike leaves with *Uromyces trifolii* was easily obtained either on distilled water or the 7 per cent sucrose solution. In the former case the pustules were smaller and paler in color than those formed on the leaves on sucrose. That alsike leaves take up sugar from solution and store it as starch was made evident by iodine tests similar to those made on the bean. Figure 2 shows the result of one such test.

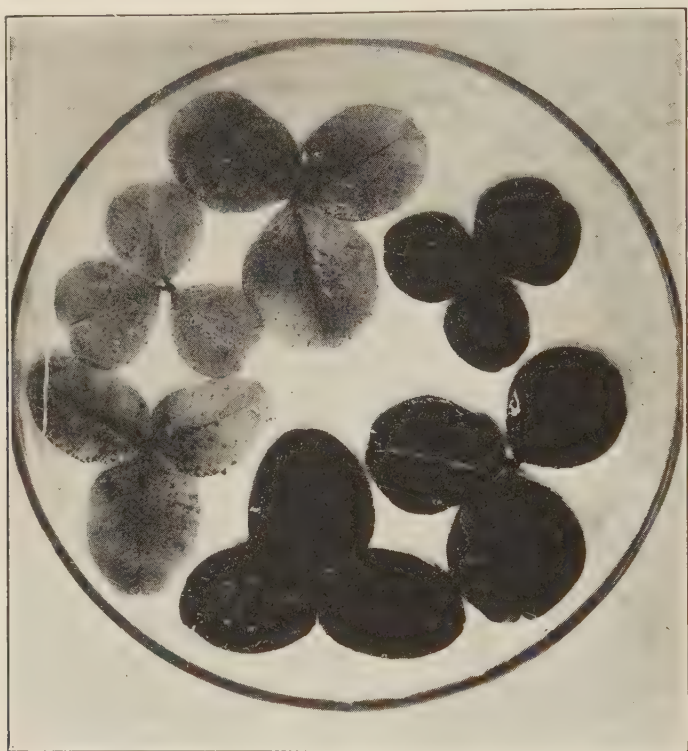


FIG. 2. Starch tests made on leaves of alsike bearing the uredinal stage of the rust. The three leaves on the left were floated on distilled water while the three on the right were floated on a seven per cent sucrose solution.

Preliminary experiments were conducted with alsike leaves by placing some of the dishes containing the leaves in the dark and others in the light during incubation. It was found that with leaves floated on sugar solutions good infections could be obtained if they were kept in total darkness during the whole period of incubation. In the case of the leaves on distilled water, however, it was necessary to place them in a well-lighted position to insure good infection. In some few cases scattered pustules were formed on those

leaves which had remained in the dark during incubation, but this can be attributed to the fact that there was a sufficient amount of stored food already in the leaves to insure at least a scanty infection. Unlike the situation found in the bean leaves, teliospores were found in only two cases out of hundreds of trials with leaves on distilled water. In these exceptions, the number of sori was very few on the leaf, in one instance numbering 20, in the other, 28. Normally, by the spray method of inoculation, 2 to 500 pustules are usually formed on a single leaf; hence it appears to be a case of the fungus famishing before being able to produce the telial stage.

To induce the formation of teliospores, various methods were tried. It has been shown in the experiments with the various host-plants in the greenhouse that exhaustion of the food supply in the host, either through lack of light or moisture, or low temperatures, seemed to inhibit further production of urediniospores with the resulting formation of the telial stage. Methods were therefore used in the petri-dish cultures to control the food supply of the host. It was found that teliospores could be induced in the leaves of alsike by three methods:

1. Starvation of the host, with the later addition of a rich food supply.
2. Sudden change of the host from a well-fed condition to a stage of starvation.
3. Continued supply of food, with the gradual dying of the host cells and lowering of metabolism.

These objectives were attained by the addition and subtraction of the nutrient solutions. The efficiency of the method is, of course, much lower in this case than in working with fungi which can be cultured directly on artificial culture media, because we have the living host, with all of its complexities of metabolism and growth, interpolated between the nutrient solution and the fungus. Nevertheless, sufficient data were secured to justify the conclusions. The attempt was also made to show the effects of low temperature on the leaf and rust metabolism as in the case of the bean leaves, but the same difficulties were encountered and that phase of the experimentation was suspended.

Table 10 shows the results of infection experiments conducted with detached leaves. The results show a relationship between the presence of carbohydrates in the leaves and successful infection.

Experiments were next conducted to show the effects of changing the nutrient solutions on the infected leaves. Alsike leaves in 32 dishes were inoculated, and at the end of 48 hours sucrose was added to 16 of them, the other 16 receiving distilled water.

Eight days after inoculation, uredinia appeared on the leaves on the sucrose solution and one day later on the leaves on distilled water. At this time seven dishes of leaves were changed from distilled water to sucrose, seven were allowed to remain on the water, six were changed from sucrose

TABLE 10.—*The effect of light and medium on development of Uromyces trifolii on detached leaves of Trifolium hybridum*

No. dishes containing detached leaves	Medium	Incubation period in days	Light condition during incubation	Infection
12	Distilled H ₂ O	7	Light	Sori small and pale in color.
8	do	10	Dark	Only two out of eight leaves infected.
8	7% sucrose solution	6-7	Light	Good infection; 200-500 pustules per leaf.
8	do	7	Dark	Fair infection, large pustules; 50-100 pustules per leaf.

to water, and ten remained on the sucrose. The solutions were changed every other day to prevent contamination.

Twenty days after inoculation the leaves were examined. The results were as follows:

1. The leaves which had been on distilled water continuously were all lightly infected, and on one leaf that had a lighter infection than the others there were teliospores formed in the old uredinial pustules and interspersed with the urediniospores. The other leaves were in a fair state of preservation, but the rust was not developing any further.

2. On the twenty-second day after inoculation teliospores were found in five of the seven dishes of leaves that had been transferred from water to sucrose. The teliospores were quite numerous and in some cases were formed in separate telia; the majority, however, were formed in the old uredinial pustules.

3. Examination of those leaves which had been on the sucrose throughout the experiment revealed only a very few teliospores. Uredinia were formed in very large amounts and the leaves had the appearance of being almost destroyed. As was found to be the case with most of the rusts, a continued supply of nourishment almost universally caused the fungus to produce the uredinial stage until finally the host was exhausted so suddenly that there was no time for the formation of teliospores.

4. The rust on leaves that had been transferred from sucrose to water had grown practically not at all after the sucrose was removed. For several days after the nutrient was taken away a few urediniospores had been formed, but not many. No teliospores were formed, and the leaves soon disintegrated.

As no teliospores formed when the leaves were transferred from sucrose to water at the time of uredinial formation, it was thought advisable to transfer them before the fungus could progress that far in its development and before it could appropriate too much of the stored food in the leaf for the production of urediniospores. Accordingly, leaves in 16 dishes were inoculated in the regular manner and set aside for only 48 hours. At the end of this time, the leaves in eight of the dishes were supplied with 7 per cent sucrose solution, the leaves in the other eight remaining on distilled water. Six days after inoculation, leaves from four of the eight dishes were rinsed and transferred from sugar to distilled water.

From four to six days later, uredinia appeared on practically all of the leaves in the 16 dishes. Twenty days after inoculation teliospores appeared on the leaves which had been transferred from sugar to water. The other leaves showed about the same results as those outlined in the preceding experiment. The fact that teliospores formed on leaves which had been transferred from sugar to water before uredinia had formed made it appear that the sudden removal of food at such a stage of development inhibited production of urediniospores and stimulated the immediate formation of teliospores.

Puccinia suaveolens Rostr., on *Cirsium arvense* (L.) Scop. *Puccinia suaveolens* was not at first readily cultured on leaves of *Cirsium arvense* in dishes due to their rapid discoloration and decomposition. In the early part of the work it seemed almost impossible to obtain infection. Finally it was found that by selecting the younger and more succulent leaves for inoculation a large percentage of them became infected.

Experiments were conducted to determine the ability of the *Cirsium* leaf to take up sugar and store it as starch in the cells, and it was found that large amounts were stored after four or five days on the 7 per cent sucrose solution.

Infections of rust on leaves floated on distilled water, if not too heavy, produced teliospores a few days after the first appearance of the uredinial stage. During winter days when the light intensity was low it was not infrequent to find that the telial stage was the first and only stage of the rust to appear on the leaves on water.

That the presence or absence of food in the leaf was a deciding factor in teliospore production is shown by the results of the following experiment. Leaves in 28 dishes were inoculated, and 48 hours after inoculation sucrose was added to 14 of them, the leaves in the remaining 14 being left on the distilled water. From five to six days later uredinia appeared on practically all of the leaves, and at this time one-half of the leaves on sucrose were rinsed and placed on distilled water, and one-half of those on water were placed on sucrose.

Fifteen days after inoculation, teliospores were found in large numbers in the old uredinial pustules of those leaves which had been continuously on distilled water; also they were being formed on those leaves which had been transferred from the sugar to the water. In the latter case they were not at all numerous and the leaves were severely injured. Various results were obtained with the leaves which had been continuously on the sugar solution. Some of the leaves had teliospores and some died after producing the uredinial stage. In all cases where the leaves remained in good condition and the infection was not heavy, teliospores were formed.

On leaves which had been originally on water and were transferred to the sugar solution, most interesting phenomena were observed. The same sori which had originally produced urediniospores and later formed teliospores again formed urediniospores when the effect of the transfer to the sugar solution was felt. The later-formed urediniospores were not very numerous but sufficient to show that by the addition of the sucrose a new food supply had been provided for uredinial production. Thus we find the following spore forms being formed successively: urediniospores, as a result of the first infection; teliospores, as a result of the distilled water for the first eight days; and lastly, the second production of urediniospores as soon as the sugar solution had had an effect. It is interesting to note that some of the leaves which were producing urediniospores for the second time were completely brown: scarcely a trace of the original green color was to be seen.

As has already been explained, the time of year with its corresponding light intensity was a deciding factor with those leaves which were put on distilled water only, and which supplied to the rust only that food which was being formed in the leaves through photosynthesis carried on while the leaves were in the dishes.

Puccinia antirrhini Diet and Holw., on *Antirrhinum majus* L. Attempts were made to culture *Puccinia antirrhini* on *Antirrhinum* leaves on solutions, but infections were so scanty that reliable conclusions could not be drawn. The leaves themselves remained in good condition in the dishes for five or six weeks either on nutrient solutions or on distilled water. Occasionally adventitious rootlets were put out from the petiole and would grow until they attained lengths of ten centimeters or more. In one instance a rooting leaf was placed in a flask with Shive's solution and remained alive for 60 days; in attempting to induce it to grow in a pot of earth, decomposition set in and it was destroyed.

The leaves were finally infected on the lower surface, as has been explained elsewhere in the paper, and from then on good infections were always obtained.

As has been noted in the work with *Antirrhinum* rust in the greenhouse, the telial stage is rare under greenhouse conditions. Likewise in the dish cultures, the uredinial stage may continue to form for long periods of time on sugar solutions and the telial stage may never appear. On infected leaves on distilled water the pustules may dry up before more than just the primary uredinial pustules can be formed.

Early in the experimental work before a successful method of inoculating the leaves had been discovered, a few experiments were conducted with cuttings in solutions. Ten cuttings were inoculated, five of which were placed with the stems in flasks of distilled water and five in a 7 per cent solution of sucrose. They were kept during the whole of the experiments under bell jars to prevent excessive transpiration. Six days after inoculation, when the first signs of uredinial production became visible but before the pustules had broken through the epidermis of the leaves, three of the cuttings in sucrose were transferred to distilled water. The others were allowed to remain in the same solutions.

On the sixteenth day after inoculation, teliospores were found on the leaves of the cuttings which had been transferred from the sucrose to the distilled water. They were not numerous and were interspersed with urediniospores in the original uredinia. There were no teliospores on the leaves of the other cuttings. The pustules on the leaves of the cuttings in the distilled water were dried up as a result of rapid starvation or desiccation. On the cuttings in the sugar solution secondary uredinia were produced in abundance, but due to contamination it was impossible to determine whether teliospores would eventually have formed or not. While these results are not in themselves especially conclusive, when taken in conjunction with the results obtained later with detached leaves on solutions they show the relationship between the available food in the host and the production of teliospores.

In experiments with the detached leaves alone, it was found that leaves which were on sugar solutions continuously, if the amount of infection was not too heavy, usually produced the greatest abundance of teliospores. Rust on leaves which were transferred from sugar to distilled water, if the transfer was made before the fungus had advanced too far in its development, generally produced the telial stage.

Table 11 gives the results of some of the experiments conducted with *C. antirrhini* on detached leaves of *Antirrhinum*.

In experiment A, leaves which were on distilled water continuously did not form teliospores because, as has been found to be the case with all of the rusts, when the infection was heavy the rust famished so quickly that the production of teliospores was impossible. In Experiment B it is seen that when the infection was light teliospores were formed even though the

leaves were on distilled water all of the time. In Experiment C the same results were obtained as in Experiment A under the same conditions. The infection in the case of the leaves on distilled water was too heavy to allow teliospore formation. The other results were similar to those obtained with other rusts with the exception that when *Antirrhinum* leaves were transferred from distilled water to sugar solutions the leaves failed to take up the sugar. As a result, in Experiment A, where the leaves were transferred from water to sugar, the rust failed to respond to the change in environment and gradually died out without producing any more spores.

TABLE 11.—*The effect of different media on teliospore formation in Puccinia antirrhini on Antirrhinum majus*

No. dishes used	First nutrient	No. days on first nutrient	Second nutrient	Incubation time in days	Formation of teliospores (no. days after inoculation)
Experiment (A)					
6	Distilled H ₂ O	10	7% sucrose	9–10	None
5	do	10	Distilled H ₂ O	9–10	do
7	7% sucrose	10	do	9–10	19
7	do	10	7% sucrose	9–10	25
Experiment (B)					
8	Distilled H ₂ O	17	Distilled H ₂ O	8	17 ^a
8	7% sucrose	21	7% sucrose	8	21
Experiment (C)					
12	Distilled H ₂ O	25	Distilled H ₂ O	10	None
14	7% sucrose	25	7% sucrose	8	25

^a Infection very light; average no. of pustules per leaf about 20.

Puccinia sorghi Schw., on *Zea mays* L. *Puccinia sorghi* was readily cultured on leaves in petri dishes and, as Mains (21) reported, good infection could be obtained in the dark when the leaves were floated on sugar solutions.

Teliospores were induced in this rust by the same general means as in the other rusts already discussed. If sucrose was added at a time when the rust was beginning to feel the effects of the diminishing food supply, teliospores were formed. They were found on leaves which were on sucrose continuously, and also on leaves which were transferred from sucrose to distilled water before the uredinial pustules were fully formed. They were never produced on leaves floated on distilled water only. It was found that, if leaves were transferred from sucrose to distilled water after the uredinia were formed, teliospores were never formed; if, however, the

transfer was made three or four days after inoculation, the telial stage always appeared. The teliospores were in all cases interspersed at first with the urediniospores until finally there were only teliospores in the pustules.

Puccinia triticina Eriksson, on *Triticum vulgare* Vill. The experiments with *Puccinia triticina* were but repetitions of the ones with *P. sorghi*, for these two rusts and hosts seemed to behave similarly. That there is a difference, however, is evidenced by the fact that teliospores of *P. triticina* are seldom, if ever, seen on young plants of wheat in the greenhouse. Mains (22) says of this rust: "*Puccinia triticina* upon wheat seedlings has never been seen to produce telia under such conditions even on old dying leaves, but does produce some telia on old leaves when the wheat plants approach maturity." Teliospores were produced on a few young corn plants in the greenhouse but they were never induced on wheat.

On the leaves in dishes the writer has been able to produce teliospores with the same ease as on corn leaves.

To avoid the contamination that is so likely when culturing wheat leaves, leaves which were already infected on the plant were used. They were taken from the infected wheat plants before the uredinia had broken through and placed upon distilled water in the dishes. After three days, one-half of the leaves were placed upon the 7 per cent sucrose solution, the other half remaining upon distilled water.

Five days later an abundance of teliospores was found upon the leaves which had been transferred to sugar. They were formed in linear sori in most cases but some were interspersed with urediniospores in the old uredinia. There were many abnormal spores: some thick-walled, one-celled with spines; others two-celled with spines; and some like the typical two-celled, smooth-walled teliospore.

As was mentioned above, wheat and corn leaves cultured in dishes were more liable to contamination than any of the leaves belonging to the dicotyledonous group. Dicotyledonous leaves have been kept in dishes for several weeks with few signs of contamination, but corn and wheat leaves usually were covered in a few days with yeasts and other contaminating fungi.

This can probably be explained by the fact that in the monocotyledons there is usually an abundance of glucose and other monosaccharides stored within the leaf while in the dicotyledons there is little sugar stored as compared with the large amounts of starch (Parkin, 32). As the monosaccharides are easily attacked by the average saprophytic fungus, it is natural to expect such leaves to be contaminated sooner than those containing insoluble starches. In placing the leaves in dishes it is sometimes neces-

sary to cut the edges, and it is at these points that there is probably a diffusion outward of the stored sugars. These regions afford a favorable place of attack for the saprophytic forms.

Puccinia taraxaci Plowr., on *Taraxacum officinale* Weber. *Taraxacum* rust is easy to culture on leaves in dishes, but the formation of the spore type desired is very difficult to control. Uredinial formation continues sometimes up to the point where the whole leaf is destroyed. In many cultures upon sucrose solutions urediniospores continued to form for seven weeks, and even up to the point where only a small fragment of the leaf remained in the dish, new urediniospores were being formed.

The rust was grown upon both sugar solution and distilled water and the usual transfers made from one to the other, but with no results other than the continued production of urediniospores or the death of the leaves. Finally it was found that by transferring infected leaves from the sugar solution to distilled water and then back again, teliospores were produced.

The leaves were first inoculated in the usual manner on distilled water and, after two days to allow for spore germination, were transferred to sucrose solution. Here they remained for 16 days, in the meantime producing secondary rings of uredinia. They were then rinsed thoroughly and placed back again upon distilled water for five days, and then finally replaced upon the sucrose solution. Four days after the last transfer, examination revealed teliospores interspersed with urediniospores in some of the secondary sori. They continued to form for several days, but later urediniospores were formed instead. These continued to form for several days until the leaves became so contaminated that they were discarded. New sets of leaves were tried, and in each case teliospores made their appearance in practically the same manner and under the same type of manipulation.

The results of these experiments, although somewhat different from those for the other rusts, show the same relationship between food condition and production of teliospores. The host requires a further state of exhaustion before the telial stage is produced. That this is true can be determined from field observation on the nature of the host and the rust. A study of this rust over a period of three years in the field has failed to reveal a single case where the telial stage has been present except in the late summer or fall. This shows that the metabolism of the host and the fungus are so closely adjusted that the telial stage is produced under only the most well adjusted conditions. Magnus (20) has noted this situation in the *Taraxacum* rust and has explained it as being due only to the fact that the host is so hardy that its metabolism is not readily upset. Quite in contrast with this rust is that upon *Cirsium* where the least unfavorable condi-

tions for the host immediately throw the spore production of the parasite from the uredinial to the telial stage. A study of the life history and habits of these two hosts, however, seems to the writer to explain fully the differences in sensitiveness of the two. Both conditions are the result of a long period of evolutionary adaptation of each rust to its particular host. The same relationship exists in each case, and it is only when the experimenter has succeeded in finding the exact condition that upsets the perfect balance between host and parasite that the rust is stimulated to produce the resistant spore. The same principle holds true in each case, the only difference being the ability to simulate the proper conditions.

Puccinia orbicula Pk. & Clinton, on *Prenanthes alba* L. Perhaps the most striking demonstration of the effects of food conditions on the type of spore produced by the rust parasite was made with *Puccinia orbicula*. On the very young plants urediniospores and teliospores accompany the aecia quite profusely if the sorus occurs adjacent to the midrib or the larger veins. Generally upon the stems there is an almost pure teliospore production. The larger and more developed leaves contain a greater percentage of urediniospores than the younger. To all outward appearances this rust is somewhat similar to the *Podophyllum* rust in the distribution of the teliospores. Just as Whetzel, Jackson, and Mains (51) termed the latter "a plastic rust," so it appears that the *Prenanthes* rust might be regarded as quite plastic.

Healthy leaves of several ages were brought in from the field and infected with material obtained from rusted plants. Dishes P1-4 contained leaves which were about two-thirds mature floated on sucrose, the margins being cut to facilitate the absorption of the nutrient. Dishes P5-8 contained large, more matured leaves, but with only the petioles immersed in the sugar solution. Dishes P9-12 contained very young leaves on distilled water. Dishes P15-18 contained very young leaves with their petioles immersed in sucrose solution, and dishes P15-18 large leaves floated on distilled water.

Four days after inoculation, the large leaves with the cut margins had become thoroughly reddened due to the formation of anthocyanin, the leaves with only the petioles immersed becoming progressively red as the solution traveled up the veins.

On June 10, or 11 days after inoculation, urediniospores appeared on the leaves in dishes P1-5. The sori were very large and quite numerous. The same day they appeared on leaves P5-8, but were not so large nor so numerous. On the next day the uredinia appeared on the remaining leaves; in the case of P9-12 they were small, few in number, and contained about 90 per cent teliospores; P13-15 showed fair infection with many teliospores



FIG. 3. Rusted leaf of *Prenanthes alba* which had been transferred from distilled water to sucrose solution after having been on the distilled water for four weeks. New uredinia are seen breaking through the epidermis around the old telia.

accompanying the urediniospores. Leaves P15-18 had a few scattered sori, but in all cases they proved to be almost wholly filled with teliospores; only a very few urediniospores had formed.

Three days later, all of the leaves were producing teliospores. On the leaves in dishes P1-4 urediniospores were produced for the longest period; on the others teliospores were found in the same ratio as given above. The experiment was abandoned with the exception of dishes P15-18. The plants in these dishes were allowed to remain on distilled water for four weeks, and then placed on sugar solution. Four days after the transfer, examination revealed the surprising fact that the leaves had become decidedly red in color and new uredinial pustules were breaking through the epidermis (Fig. 3). The pustules could only have been the result of mycelia that had been dormant in the leaf for some time; and the sudden addition of the nutrient had renewed growth so that uredinia were produced. It must be remembered that these were the same leaves that four weeks earlier had shown only telial formation on the distilled water.

The dishes had remained closed for the preceding few weeks and there was little chance that the infections were the result of urediniospores from the outside. To the writer the logical conclusion appears to be that the type of spore which had been first produced and that which was later produced was determined wholly by the presence or absence of the carbohydrate within the cells of the leaf.

DISCUSSION

General Principles

It is evident that there exists between the host and its rust parasite a very close and intimate relationship, with the life processes of the two so closely attuned that a slight disturbance in the metabolism of the former is sufficient to disturb the latter and cause it to pass from a stage of active fructification, as evidenced by the uredinial generation, to one of quiescence, the telial. In extreme cases the uredinial stage may be entirely suppressed and then only the telial stage appears.

That the host does possess a very direct influence upon the fungus has been shown by Arthur (3), who points out very clearly that, as a rule, the vigor of the parasite is directly proportional to the vigor of the host; and that to be successful in culturing the rust fungus on its host it is necessary to use strong, rapidly growing plants. Stakman (41) believes that whatever favors the normal development of the host is ordinarily conducive to the vigorous development of the parasite. Ward (49) found that a starved host meant necessarily a starved rust, while Stakman and Levine (43) have shown that environmental conditions unfavorable to the host were also unfavorable to the rust. That the virulence of the disease may be directly

correlated with the "vegetative vigor" of the host has been pointed out by Raines (33). Sheldon (38) concluded that the lowered vitality of the host does not favor infection and anything affecting the growth of the host also affects in like manner the rust.

With the opinions and results of these investigators in mind, let us consider for a moment the source and nature of the food of the fungus, and see whether the above conclusions and those to follow do not show definitely that the rust fungus is dependent upon the photosynthetic activity of the host; and that any factor, or set of factors, such as temperature, light, moisture, etc., or a complex of these factors, such as climate, will so influence the metabolic condition of the host that it will be reflected in the type of spore produced by the rust. Until we can definitely separate the rust fungus from its obligate association with the host, and study the direct relationship of the fungus to such environmental factors as light, temperature, etc., while culturing it under strictly saprophytic conditions, we are not justified in assigning specific external influences to it. We are compelled to consider the rust fungus in terms of its most influential environment, viz., the host.

To determine the dependence of the rust fungus upon the photosynthetic activity of wheat seedlings inoculated with *Puccinia graminis*, experiments were made by Stakman and Levine (43). They concluded that "Inasmuch as the photosynthetic activities of the host plant are affected by the light intensity, in so much does the structure and function of the rust depend on the same factor."

That the rust is dependent upon intermediate products of photosynthesis was advocated by Fromme (12), while Mains (21) suggested that it is probably dependent upon some transitory or nascent organic products related to the carbohydrates.

Similar evidence of the dependence of the rust upon the photosynthetic activity of the host was obtained by the writer (50) in the case of bean rust, *Uromyces appendiculatus*, and in the present experimental work in ten additional species of rusts. It has been found in all the experiments conducted that once the parasite has gained entrance to a susceptible and congenial host, those conditions which guarantee an optimum supply of carbohydrate food for the host likewise insure a vigorous development of the fungus, as evidenced by a copious production of urediniospores. If, however, through the influence of internal or external factors, or a complex of such factors, the host is no longer able to maintain this condition of vegetative vigor, coincident with its ability to increase and maintain the carbohydrate content of its cells, then the fungus will, if still able to function, react to such conditions, and produce the resistant or telial stage.

Iwanoff (18), who attempted experimentally to prove the direct effect of climate upon certain rusts, illustrated this point in an irrefutable manner. He found that when he transported infected plants from regions of low altitudes to alpine stations he obtained a suppression of the uredinal stage with the hastening of the telial generation. He obtained the same results when he placed infected plants in an ice-chest in the regions of low altitude. The writer also produced the telial stage in seven species of rusts by the latter method. In the case of bean rust, when infected plants bearing uredinia were placed in a cold chamber at a temperature of 7° C. for two days, the telial stage appeared 13 days earlier than on the checks which remained in the greenhouse. Similar results were obtained in the other six species.

Morganthaler (28) transported plants of *Veratrum album* L., infected with *Uromyces veratri* (DC.) Schr., to alpine regions and found that the telial stage was produced sooner than in those plants which remained at low altitude.

Now, do any of these results justify the conclusion that the climate as such or the temperature in itself was directly influential in producing the telial stage? Is it logical to assume that the bean host represents the same set of food conditions at 7° C. as it did at 20° C? In a similar manner, can Iwanoff say that the same rate of metabolism was going on in his host plants at the plains station at an average temperature of 20° C. and an altitude of 520 meters, as was present at the alpine station Faulhorn at an altitude of 2684 meters and a temperature ranging from 2 to 28° C.? Morganthaler took account of these questions and concluded from his experiments that the climate was not directly responsible, but that the change in the altitudinal position of the host plants affected their nutrition, probably retarding it, and in this way induced the formation of the teliospores. He furthermore demonstrated that, by cutting the veins of leaves and by detaching or singeing leaves of *Veratrum album* infected with *Uromyces veratri*, he could induce the telial stage of the rust. He thus concludes that he is not far wrong in assuming that the principal cause for teliospore formation is the diminishing of food; that teliospores assume the role of resistant spores in the life history of the rusts and that the taking away of food stimulates their appearance.

Gassner (14) likewise was unwilling to ascribe to climatic influence the simple direct action that Iwanoff suggests. In his opinion the climate always acts upon the rust in a roundabout way through the host. He showed through extended observations and experiments that, under normal conditions of growth, the time of appearance of the telial stage of grain rusts has a certain relation with the stage of development of the host plant.

This usually occurs at the time of, or just preceding, the heading out of the grains, when there is a general mobilization of food within the plant for seed production. This stage he called the point of exhaustion of the host and assumed that at this stage the diminution of the food caused the fungus to produce the telial stage. He also showed that different hosts reacted differently in that stage of exhaustion. This was illustrated by *Puccinia triticina* and *P. graminis*; he succeeded in obtaining the uredinial stage of the latter upon hosts which were already bearing the telial stage of the former. It appears, however, that Gassner, although he was close to the fundamental issue of the matter, associated too closely the developmental stage of the host with telial production. Practically all his experimental work was done in the field, where environmental conditions other than purely climatic were not operative. In only two or three instances does he mention any attempt to use experimental means to hasten or retard telial formation. In the one case he cut notches in the leaves of the rusted hosts but with no results, the leaves dying soon after. The fungus on starved plants grown on quartz sand failed to produce the telial stage. By decreasing the light he did succeed in inducing the formation of teliospores on infected oat plants sooner than on the checks.

It would seem that even though, in the grain rusts, the telial stage usually accompanies a certain stage of development of the host, as Gassner has shown, it is only that the food situation at that time is in a state of mobilization for seed production and as a result the fungus is cut off from its food supply. The condition, under which the fungus suffers from nourishment inadequate for urediniospore production, may be brought about in a number of ways, so that the particular phase of the story brought out by Gassner is but one angle of the situation. The writer produced these same conditions by subjecting the infected hosts, without regard to age or stage of development, to low temperatures, lack of light, insufficient moisture, and by floating detached leaves upon various nutrient solutions. It is quite natural that under field conditions the telia would appear normally at a certain stage of development of the host; but the same host, under varying conditions of environment, may be so affected as to produce the telial stage at any or at all periods in its life history.

This is illustrated in the case of *Puccinia sorghi*. Gassner (14) found that on plants of *Zea mays* the telial stage appeared at about the time of flowering, *i.e.*, when the plants were about three or four months old. In the present paper it is reported that teliospores were produced on corn plants which were only 30 days old, and which were exposed to low temperatures for only one day. In this case it is evident that the stage of development of the host was not important.

The experiments described in the present paper are but a few among many that could be conducted to illustrate this fact. To bring about the proper balance of food supply between the fungus and the host to produce the telial stage is a problem that depends entirely on the experimental technique and that only continued trials will solve. This is seen in the cases of the different hosts used in the temperature experiments. In the behavior of the *Cirsium* and *Taraxacum* rusts the same principle is involved, with the same outcome in both cases; but to bring about the same results in the two hosts a different procedure is required. As mentioned previously, when *Cirsium* leaves were placed in the cold room for five days the telial stage was produced eight days sooner than on the checks. In the case of *Taraxacum*, placing the infected plant under the same conditions for seven days failed even to stop uredinial production. Three days in a similar environment completely killed the corn plant. Thus, it is evident that the structure and habit of the host plant must be taken into consideration as well as the environment to which it is accustomed. Having discovered the conditions under which the metabolism of the host can be regulated, it is safe to assume that the rust will correspondingly reflect the varied conditions in the type of spore which it will produce.

In attempting to interpret the results of the temperature experiments with such seemingly divergent rusts as those on *Taraxacum* and *Cirsium*, we must consider the nature of the two hosts as found under field conditions. *Cirsium* grows very rapidly: its life processes are carried through in exceedingly rapid succession. In three weeks a plant two or three inches in height may become several feet tall and have fully developed flowers. Under such conditions it is evident that there must be speedy assimilation and translocation of food. The rate of metabolism of the leaves becomes progressively slower as the tissues rapidly mature, and to a parasite inhabiting the leaves of such a plant the food becomes quickly unavailable. What is the result? As one would expect, either the fungus becomes adapted to such conditions and is capable of quickly protecting itself or else it is exterminated. Such protection comes through the formation of teliospores. These are produced at the first sign of waning metabolism, and by the time the food is completely diminished the telial stage is formed. Likewise, under experimental conditions, when the infected host is subjected to any abnormal environment retarding its metabolism, the rust will quickly respond by producing teliospores. Hence, when *Cirsium* leaves were placed under the influence of low temperature, absence of light, or when the detached leaves were transferred from sugar solution to distilled water, the results were identical with those obtained when the host approaches maturity in the field. Similarly, the telial generation is produced when leaves are placed continuously upon

distilled water if, on such leaves, there is a light infection. Ordinarily, on heavily infected leaves, which obtained no nutrient other than that which was synthesized within the dishes, no teliospores were produced. In other words, the fungus starved before telia could be formed. A similar case has been referred to by Gassner in answering the objections of Lagerheim, who maintained that the telial stage does not appear with the exhaustion of the host plant, as he had observed wilted plants of *Vicia faba* L. infected with *Uromyces fabae* de Bary in Quito, Ecuador, on which only urediniospores were visible. Gassner points out that this is probably due to the fact that the plants had wilted suddenly and were not able to reach maturity. He uses this example to point out that wilting and exhaustion are not identical. However, if Gassner had changed experimentally the environmental conditions of this same host he probably would have found that even as wilting and exhaustion are not identical conditions, neither is exhaustion of the host associated necessarily with its later or maturing stage of development.

In the case of the *Taraxacum* rust, where we find apparent differences in reaction, closer analysis reveals the same underlying influence of nutrition. Under the ordinary methods of greenhouse culture no teliospores were ever produced on the *Taraxacum* plant, for the uredinial stage persisted until the death of the infected host parts. But, when the host was subjected to successive exposures of low temperature, the telial generation made its appearance, as demonstrated in plants T10-15.

Taraxacum grows rather slowly in Michigan. The leaves are numerous and grouped closely upon a central axis, with no necessity for rapid assimilation or translocation of elaborated food over long distances. The plant may be found growing more or less actively in almost any month of the year. One would expect the food situation in such a plant to be stable, with no such rapid fluctuations as occur in *Cirsium*. In such a host the sudden diminution of available food, at least during the growing season, is not felt by the rust parasite. It is only with the advent of short days and low temperatures, when there is a gradual withdrawal of food from the leaves of the host, that the fungus is stimulated to produce the telial stage. The life processes of the host and fungus are so in accord that the latter is a direct result of the former. Any treatment that will bring about this unfavorable condition of food in the host will cause the fungus to produce the telial stage. Such a condition was produced when *Taraxacum* was subjected to alternate periods of low temperature and absence of light. Afterwards, when the host was returned to a favorable environment, the rust resumed its formation of the uredinial generation.

The same correlation between the type of host employed and the method

of manipulation required to produce the telial stage of the rust inhabiting such a host is seen in petri-dish culture.

In the case of *Cirsium*, teliospores appeared on the leaves in the dishes under several sets of conditions. If the leaves floated on distilled water were not too heavily infected, the telial stage appeared soon after the first appearance of the uredinia. Likewise, if the leaves were transferred from the 7 per cent sugar solution at the time of uredinial production to distilled water, the telial stage appeared. On the leaves which were continuously on sugar, providing the infection was not too heavy, teliospores formed also, but only after a longer period of time. It is possible that if the leaves could be so kept that they would continue to absorb the carbohydrate gradually, the uredinial stage would persist for much longer periods of time. In spite of all precautions, it is probable that the outer layers of cells adjacent to the cut surfaces die either as a result of bacterial action or autolysis, and further absorption ceases. In such cases, of course, telial formation occurs.

Another explanation which might be presented to explain this cessation of uredinial development on the leaves is that the rusts may not exist upon a purely elaborated carbohydrate diet but require some products related to the proteins. In such an event, when the leaf is left continuously upon the sugar solutions its cells would soon be depleted of such mineral nutrients as nitrogen, potassium, etc., and the further assimilation by the host of food suitable for the fungus would cease. Even the fact that the rust thrives for a time upon a leaf so nourished does not preclude the possibility of a partial protein-related diet. In an ordinary detached leaf there undoubtedly exists sufficient amounts of nitrogen, potassium, etc., within the cells to insure the normal development of the rust for a considerable period. The experiments of Ward (49) and Mains (21) showed that even when the host plant was starved with respect to certain mineral elements, infection could still take place.

To induce teliospore formation upon the leaves of *Taraxacum*, as is shown in the results of the experiment, it was necessary to make several changes in the medium upon which the leaves were floated. They were first floated upon distilled water, then immediately after inoculation changed to sugar solution for 16 days. At the end of this time they were transferred to distilled water for five days, after which they were returned to the sugar solution.

At the time of the transfer from sugar to distilled water the rust was producing an abundance of uredinia. This would mean that a great amount of food was being taken up from the sugar solution by the leaf and appropriated by the fungus. The sudden change to distilled water cut off this continuous food supply and stimulated the fungus to initiate the produc-

tion of the telial generation. However, due to the insufficient supply of stored food both in the leaf cells and in the mycelium, the fungus could not carry through to completion the formation of the telial stage. This was shown when the leaves were allowed to remain on distilled water at this stage of the experiment. If, after a period of five days the leaves were again transferred to the sugar solution, adjustments already initiated due to the stimulus were carried over and the fungus completed the formation of the telial stage. Soon after, the effects of the addition of the nutrient to the leaf cells again caused the fungus to resume uredinial production.

The Light Relations

With regard to the influence of light upon the growth and development of the rust mycelium, little need be said, for Fromme (12), Mains (21), and the writer (50) have shown that, in the organisms employed by them, the main influence is exerted indirectly through the host. We can consider that any results obtained from the experiments reported in this paper represent the effects of light acting not especially upon the fungus, but mainly and, perhaps only, through the host.

In five of the organisms studied, namely, *Uromyces appendiculatus*, *Puccinia suaveolens*, *U. trifolii*, *U. polygoni*, and *P. sorghi*, it was possible to inhibit the uredinial stage and hasten the time of telial formation by placing the infected plant in the dark for varying periods of time. The time of appearance of teliospores varied up to a certain limit directly with the length of time the host was left in darkness. Since the temperature was practically the same as for checks in the light, the effects produced could only have been due to the decrease in photosynthetic activity of the host, with the resulting partial starvation of the fungus.

It must not be assumed, however, that mere starvation and injury to the host in all cases will guarantee the appearance of the telial generation. It is undoubtedly true that the diminution of food will initiate the formation of such a spore stage, but in all cases the fungus must have access to enough nutrient to carry through to completion the reaction to such a stimulus. This was shown in the case of corn rust. When the host was subjected to total darkness for either three or four days, both host and parasite died before the telial generation was formed. Two days, however, under the same conditions produced the necessary "starvation" stimulus and, when the host was again removed into the light to the greenhouse, it was capable of synthesizing just enough food to enable the fungus to complete the formation of the telial stage. If the metabolism of the host and fungus are not too greatly disturbed, it should be possible, in some cases, to induce uredinial formation again following the telial stage. This is what actually occurred in the case of *Uromyces polygoni*. When plants

P1-5 and P5-10 were removed from the cold, dark room (7° C.), and the dark room (19° C.), respectively, after having been there for four days, the telial stage was first produced; then, under the influence of the favorable conditions of the greenhouse, the uredinial generation was again produced. When, for the second time, the plants were subjected to the same conditions of light and temperature as before, the fungus proceeded to produce the telial stage again. This time, however, plants P1-5 were not able to respond to the resumed favorable conditions and the fungus continued to produce the telial stage until the death of the infected leaves. On P5-10, which had not been so severely affected, uredinial production was resumed for a short time before finally the telial stage appeared.

Several of the other rusts reacted in a similar fashion, and the results appear so positive that discussion is not deemed necessary to point out further the direct connection between the presence or absence of food and the type of spore produced.

Moisture Relations

The experiments conducted relative to moisture control seem to show that, in certain rusts at least, the amount of moisture available for the host and parasite, be it soil or atmospheric moisture, exerts an influence on the type of spore produced by the rust parasite.

The conclusions drawn by the writer from the results of experiments conducted with asparagus rust are perfectly in accord with those of Smith (40), as far as the assumption goes that teliospores are produced when moisture conditions, be it soil or atmospheric, are unfavorable for the further vegetative development of the fungus. The lack of agreement comes in the interpretation of the manner in which the moisture affects the fungus.

Smith assumes that an abundance of soil moisture favors the host by giving it increased vitality and resistance with the result that the fungus is retarded and forced to form the telial stage. This is clearly at variance with the findings of such workers as Arthur (3), Stakman (41), Ward (48), Stakman and Levine (43), *et al*, mentioned at the beginning of the discussion. However, as Smith's conclusions are based on field observations, it appears to the writer that there are too many unknown factors which may have entered in to ensure the absolute accuracy of his interpretations.

Sheldon (37, 38), was forced to disagree with Smith's interpretations, for he concluded that, as a rule, conditions favorable for the development of the host were also favorable for the development of the fungus. The same conclusions were reached by the writer, with an added feeling of assurance that an abundance of soil moisture not only increases the vitality of the host but also lengthens the period of uredinial production by the rust.

When plants A5-6 were placed in the greenhouse and kept well watered, they continued to produce the uredinial stage for a week longer than did plants A1-5, which were deprived of their soil moisture.

If the conclusions of Smith be correct, then the presence of abundant soil moisture should have rendered the host an unfavorable medium for the fungus and the telial stage should have appeared sooner than on plants A1-5. This, however, was not the case. On the contrary, the results seemed to show that the abundance of soil moisture exerted a favorable effect on the host and also on the fungus, due to the increased ability of the former to produce an abundance of food.

With respect to the action of atmospheric moisture, Smith (40) asserts that "atmospheric dryness checks aecidial development and uredo-development, and changes to a production of teleutospores in the sori already formed, without regard to season or conditions of the host plant. With moisture, uredospore formation begins again at once."

It is difficult to understand how such a statement can be positively made, for such an assertion disclaims any influence that the living host might exert at the same time. Unless Smith can show that the asparagus rust parasite fails to maintain that close association with the host which is common to so many of the rusts, he must admit that the condition of the host should play an exceedingly important rôle. Can he maintain that the host, under the influence of high humidity, is, physiologically speaking, the same host upon which the telial stage was being produced? Is it not true that the increase in humidity would tend to lessen the transpiration and evaporation from the infected stalks with the result that the metabolic rates of both the fungus and the host would remain lower and the cells of the host remain alive for a longer period of time? Would not this in itself affect the food relations of the fungus?

Resistance of the Host

Fromme and Wingard (13) found a relationship between the resistant varieties of bean and teliospore formation. When certain resistant varieties of bean were inoculated, teliospores, or mixed sori, made their appearance immediately.

In working with *Puccinia coronata* Corda, on *Avena sativa* L., Parker (31) observed that frequently the telial stage appeared on seedling leaves of resistant varieties, while on the susceptible varieties it did not occur.

In table 3 it is seen that on the Scarlet Wax variety of bean, which is resistant to rust, teliospores were formed 15 days after inoculation, or only three days after the first appearance of the uredinial stage. In some cases the teliospores appeared without being preceded by this stage.

It is impossible to analyze all the possible factors in resistant and susceptible plants, as no one has yet satisfactorily explained what the phenomenon of resistance actually means. The cytology of infection, both in resistant and susceptible plants, has been investigated by several, among whom are Ward (47, 48), Evans (11), Stakman (41, 42), and Allen (2). In all cases they have shown that, when a rust infects a host, vigorous development takes place without any immediate serious injury to the host. In fact, the host cells seem to be stimulated to greater activity for a while. In resistant hosts, however, the host cells in the immediate vicinity of the haustoria are killed and the fungus makes little headway. The extent to which this killing takes place seems to depend upon the degree of resistance of the host. Whether, as Marrrayat (24) has declared in the case of *Puccinia glumarum*, the fungus starves as a result of the death of the host cells, it is difficult to say; but, at any rate, from all the cytological evidence presented, it is certain that the fungus in a resistant host is not able to establish a satisfactory food relationship with the host. Such being the case, it is entirely possible that this condition of partial starvation would immediately stimulate the production of teliospores as was seen in the bean.

Since, as has been pointed out in all the above experiments, the appearance of the telial stage is an index of some metabolic disturbance within the host, it is entirely within reason to suppose that the early appearance of the telial stage on a resistant plant is due to the inability of the fungus to establish a satisfactory food relationship with the host.

SUMMARY

1. From the results of the experiments herein described, it appears that all the rusts studied are directly dependent upon the photosynthetic activity of the host. Any single factor or set of factors, such as light, temperature, and moisture, or, as in the case of climate, a complex of these factors, may so influence and do influence the metabolism of the host, that the fungus reacts by changing from the uredinial to the telial generation, or, under proper manipulation, in the reverse direction.

Results obtained in the greenhouse

2. Nine species of rusts were cultivated in the greenhouse and experiments conducted to show that teliospore production can be controlled.

3. With the exception of *Puccinia triticina* on *Triticum vulgare*, teliospores were formed in all the rusts when the host plants were placed under various environmental conditions unfavorable for their metabolism.

4. In seven of the rusts, teliospores were produced when the infected hosts were placed in darkness at 7° C., or in darkness at 19° C.; in the

former environment teliospores appeared several days sooner than in the latter.

5. The conditions of light and temperature necessary for production of the telial stage vary with different hosts. Such conditions appear to be governed largely by the nature and habits of growth of the host.

6. In three of the rusts, teliospores were produced when the infected hosts were slowly deprived of water, the amount and general procedure varying with the nature and habit of the host.

7. Resistance of the host plays a part in the production of teliospores. In the case of bean rust (when the host was partially resistant) the telial stage was formed several days earlier than when it was susceptible. In some cases the uredinial generation was completely inhibited and teliospores were the first structures to appear following infection.

8. By removing the growing point of the bean plants immediately following inoculation and thus preventing the withdrawal of food from the primordial leaves, the time of teliospore formation was postponed several days.

Results obtained in petri dishes

9. A method was devised by which detached leaves or portions of leaves of the host plant could be infected and floated on water or nutrient solutions in petri dishes. By this method infections were obtained regularly and in some cases they exceeded in amount those obtained on plants in the greenhouse.

10. Ten rusts, including the nine used on potted plants, have been cultured by the above method. The additional rust was *Puccinia orbicula* Pk. and Clint. on *Prenanthes alba* L.

11. Urediniospores and teliospores were produced in eight of the rusts which were cultured in dishes, the type of spore formed depending on the treatment to which the leaves of each host were subjected.

12. The rusts in which the telial generation was produced most easily in the greenhouse were also the ones which responded most readily to experimental methods in dishes.

13. When infected bean leaves were transferred at the time of uredinial formation from a 7 per cent cane-sugar solution to distilled water, if the infection were not too heavy, teliospores were produced much sooner than on leaves which were allowed to remain on the sugar solution continuously.

14. On infected bean leaves, grown on distilled water, teliospores appeared greatly in advance of those on leaves which were better nourished.

15. The heaviest infection on bean leaves was obtained by transferring the leaves from distilled water to sugar solution six days after inoculation.

16. Starch tests made on bean leaves at the time of uredinial and telial formation showed an abundance of starch at the time of uredinial formation and a paucity of starch during the production of telia.

17. In the case of most of the rusts cultured in petri-dishes, teliospore formation was stimulated by any one of the three following methods: (a) starvation of the host with the later addition of a rich food supply, (b) sudden transfer of the host leaves from a well-fed condition to a state of starvation, (c) continuous supply of food with the gradual dying of the host cells.

18. In two of the rusts the telial generation was induced only by transferring the leaves from the sucrose solution to distilled water and back again.

UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN.

LITERATURE CITED

1. ACTON, E. H. The assimilation of carbon by green plants from certain organic compounds. *Proc. Roy. Soc. London* 47: 150-175. 1889.
2. ALLEN, RUTH. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms III and XIX. *Jour. Agr. Res.* 26: 571-604. 1923.
3. ARTHUR, J. C. Problems in the study of plant rusts. *Bul. Torrey Bot. Club* 30: 1-18. 1903.
4. ———. Terminology of the spore-structures in the Uredinales. *Bot. Gaz.* 39: 219-222. 1905.
5. ATKINS, W. R. G. *Researches in Plant Physiology.* 328 pp. New York. 1916.
6. BOEHM, J. Ueber Stärkebildung aus Zucker. *Bot. Zeit.* 41: 32-38, 49-54. 1883.
7. CARLETON, M. A. Culture methods with Uredineae. *Jour. Appl. Micr. and Lab. Meth.* 6: 2109-2114. 1903.
8. CLINTON, G. P., and F. A. McCORMICK. Rust infection of leaves in petri-dishes. *Conn. Agr. Exp. Sta. Bul.* 260. 1924.
9. CZAPEK, F. Der Kohlenhydrat-Stoffwechsel der Laubblätter im Winter. *Ber. Deut. Bot. Gesell.* 19: 120-127. 1901.
10. DORAN, W. L. Rust of *Antirrhinum*. *Mass. Agr. Exp. Sta. Bul.* 202. 1921.
11. EVANS, J. E. P. The cereal rusts. I. The development of their uredo mycelia. *Ann. Bot.* 21: 441-466. 1907.
12. FROMME, F. D. The culture of cereal rusts in the greenhouse. *Bul. Torrey Bot. Club* 40: 501-521. 1913.
13. ———, and S. A. WINGARD. Varietal susceptibility of beans to rust. *Jour. Agr. Res.* 21: 385-404. 1921.
14. GASSNER, GUSTAV. Die Teleutosporenbildung der Getreiderostpilze und ihre Bedingungen. *Ztschr. Bot.* 7: 65-120. 1915.
15. HACKEY, J. F. Germination of teliospores of *Puccinia antirrhini*. 13th Ann. Rept. Quebec Soc. Protect. Plants. 54-57. 1921.
16. HENNINGS, P. Anpassungsverhältnisse der Uredineen bezüglich der physikalischen Beschaffenheit des Substrates. *Hedwigia.* 40: 125-128. 1901.
17. HUNT, N. REX. The "iceless refrigerator" as an inoculation chamber. *Phytopath.* 9: 211-212. 1919.

18. IWANOFF, B. Untersuchungen über den Einfluss des Standortes auf den Entwicklungsgang und den Peridienbau der Uredineen. *Centralbl. Bakteriologie. Abt. II.* 18: 265-274. 1907.
19. LIDFORSS, B. Zur Physiologie und Biologie der wintergrünen Flora. *Bot. Centralbl.* 68: 33-44. 1896.
20. MAGNUS, P. Ueber das Auftreten der Stylosporen bei den Uredineen. *Ber. Deut. Bot. Gesell.* 9: 85-92. 1891.
21. MAINS, E. B. The relation of some rusts to the physiology of their hosts. *Amer. Jour. Bot.* 4: 179-220. 1917.
22. ———. Notes on greenhouse culture methods used in rust investigations. Reprint from *Proc. Ind. Acad. Sci.* 33: 241-257. 1923.
23. ———. Notes on the life history of the snapdragon rust, *Puccinia antirrhini* Diet. & Holw. *Phytopath.* 14: 281-287. 1924.
24. MARRYAT, DOROTHEA. Notes on the infection and histology of two wheats immune to the attacks of *Puccinia glumarum*, yellow rust. *Jour. Agr. Sci.* 2: 129-138. 1907.
25. MELCHERS, L. E. A way of obtaining an abundance of large uredinia from artificial culture. *Phytopath.* 5: 236-237. 1915.
26. MELHUS, I. E. Culturing of parasitic fungi on the living host. *Phytopath.* 2: 197-203. 1912.
27. MEYER, ARTHUR. Bildung der Stärkekörner in den Laubblättern aus Zuckerarten, Mannit und Glycerin. *Bot. Zeit.* 44: 81-88, 105-113, 129-137, 145-150. 1886.
28. MORGENTHAUER, O. Ueber die Bedingungen der Teleutosporenbildung bei den Uredineen. *Centralbl. Bakt. Abt. II.* 27: 73-92. 1910.
29. MUDGE, C. S. The effect of sterilization upon sugars in culture media. *Jour. Bact.* 2: 403-415. 1917.
30. OLIVE, E. W. Intermingling of perennial sporophytic and gametophytic generations in *Puccinia podophylli*, *P. obtegens* and *Uromyces glycyrrhizae*. *Ann. Mycol.* 11: 295-311. 1913.
31. PARKER, J. H. Greenhouse experiments on rust resistance of oat varieties. *U. S. Dept. Agr. Bul.* 629. 1918.
32. PARKIN, JOHN. Contribution to our knowledge of the formation, storage and depletion of carbohydrates in monocotyledons. *Trans. Roy. Soc. London. Series B.* 195: 35-79. 1899.
33. RAINES, M. A. Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants. *Amer. Jour. Bot.* 9: 183-203, 215-238. 1922.
34. ROSTRUP, E. Ein eigenthümliches Generationsverhältniss bei *Puccinia suaveolens* (Pers.) Rostr. *Bot. Zeit.* 32: 556-557. 1874.
35. SAPOSCHNIKOFF, W. Die Stärkebildung aus Zucker in den Laubblättern. *Ber. Deut. Bot. Gesell.* 7: 258-260. 1889.
36. ———. Ueber die Grenzen der Anhäufung der Kohlenhydrate in den Blättern der Weinrebe unter anderer Pflanzen. *Ber. Deut. Bot. Gesell.* 9: 293-300. 1891.
37. SHELDON, J. L. Preliminary studies on the rusts of the asparagus and carnation. *Science* 16: 235-237. 1902.
38. ———. The effect of different soils on the development of the carnation rust. *Bot. Gaz.* 40: 225-229. 1905.
39. SHIVE, J. W. A three salt nutrient solution. *Amer. Jour. Bot.* 2: 157-160. 1915.
40. SMITH, R. E. Water relation of *Puccinia asparagi*. *Bot. Gaz.* 38: 19-34. 1904.

41. STAKMAN, E. C. A study in cereal rusts; physiological races. Minn. Agr. Exp. Sta. Bul. 138. 1914.
42. ———. Relation between *Puccinia graminis* and plants highly resistant to its attack. Jour. Agr. Res. 4: 193-200. 1915.
43. ———, and M. N. LEVINE. Effect of certain ecological factors on the morphology of the urediniospores of *Puccinia graminis*. Jour. Agr. Res. 16: 43-77. 1919.
44. TOLLENAAR, DIRK. Omzettingen van Koolhydraten in het Blad van *Nicotiana tabacum* mit Referat: Der Kohlenhydratstoffwechsel im Laubblatt von *Nicotiana tabacum*. Het Laboratorium voor Landbouw-Scheikunde en het Laboratorium voor Plantenphysiologisch Onderzoek. No. 12. 1925.
45. TUBEUF, K. F. VON. Diseases of plants. English Edition. 598 pp. London, New York, and Bombay. 1897.
46. WARD, H. M. Illustrations of the structure and life history of *Puccinia graminis*, the fungus causing the "rust" of wheat. Ann. Bot. 2: 207-222. 1888.
47. ———. The Bromes and their rust fungus (*Puccinia dispersa*). Ann. Bot. 15: 560-562. 1901.
48. ———. On the relations between host and parasite in the Bromes and their brown rust, *Puccinia dispersa* (Erikss.). Ann. Bot. 16: 233-315. 1902.
49. ———. Experiments on the effect of mineral starvation on the parasitism of the uredine fungus, *Puccinia dispersa*, on species of *Bromus*. Proc. Roy. Soc. London 71: 138-151. 1902.
50. WATERS, C. W. The reaction of bean rust grown on leaves in solutions. Papers Mich. Acad. Sci. 5: 163-177. 1925.
51. WHETZEL, H. H., H. S. JACKSON, and E. B. MAINS. Composite life history of *Puccinia podophylli* Schw. Jour. Agr. Res. 30: 65-79. 1925.

